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

I Background Information:

A 510(k) Number

K253316

B Applicant

PhAST Corp.

C Proprietary and Established Names

PhAST instrument and PhAST Blood Culture Gram-negative Panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SAN	II	21 CFR 866.1650 - A Cellular Analysis System For Multiplexed Antimicrobial Susceptibility	MI - Microbiology
LON	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 PhAST instrument and PhAST Blood Culture Gram-negative Panel (PhAST BC GN Panel) for testing positive blood culture samples for qualitative ("breakpoint" device) determination of antimicrobial susceptibility testing (AST) of specific antimicrobials with specific gram-negative organisms
- To establish a Predetermined Change Control Plan (PCCP) for removal of existing limitations imposed due to insufficient resistant isolate testing.

B Measurand:

Antimicrobial		Categorical Interpretations*
Amikacin	AMK	S, I, R
Ampicillin/sulbactam	SAM	S, I, R
Aztreonam	ATM	S, I, R

Cefepime	FEP	S, SDD, R
Ceftazidime	TAZ	S, I, R
Ceftazidime/avibactam	CZA	S, R
Ceftriaxone	CRO	S, I, R
Ciprofloxacin	CIP	S, I, R
Ertapenem	ETP	S, I, R
Gentamicin	GEN	S, I, R
Levofloxacin	LVX	S, I, R
Meropenem	MEM	S, I, R
Piperacillin/tazobactam	TZP	S, I, R
Tobramycin	TOB	S, I, R
Trimethoprim/sulfamethoxazole	SXT	S, R

*S – Susceptible; I – Intermediate; SDD – Susceptible-Dose Dependent; R – Resistant

C Type of Test:

Qualitative antimicrobial susceptibility test (AST) system that utilizes single-cell video microscopy and analysis of single-cell phenotypes of organisms in positive blood culture samples to determine susceptibility based on categorical interpretation.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use.

B Indication(s) for Use:

The PhAST Blood Culture Gram-negative Panel (PhAST BC GN Panel) is an *in vitro* diagnostic assay for qualitative (breakpoint) determination of antimicrobial susceptibility testing (AST) of pathogenic gram-negative bacteria from positive blood cultures (BC) and is intended to be used with the PhAST instrument. The PhAST BC GN Panel is a phenotypic test that utilizes video microscopy and analysis of single-cell phenotypes to determine susceptibility based on categorical interpretation. The PhAST BC GN Panel does not provide organism identification. The PhAST BC GN Panel is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system and reported as monomicrobial gram-negative specimens by Gram staining. Organism identity must be entered into the PhAST instrument or PhAST web application before categorical interpretation (Susceptible, Susceptible-Dose Dependent, Intermediate, Resistant, S/SDD/I/R) of antimicrobial susceptibility is reported. The PhAST BC GN Panel contains 15 antimicrobials.

The PhAST BC GN Panel is intended to aid in the diagnosis and treatment of individuals suspected of bloodstream infection by a healthcare provider. Results should not be used as the sole basis for patient management decisions.

This test is performed by laboratory health professionals in a clinical diagnostic setting. Results may be used as an aid to clinicians in determining appropriate antimicrobial therapy. Test results from the PhAST BC GN Panel should be interpreted 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. Sub-culturing is necessary to support further testing for: bacteria and antimicrobials not on the PhAST BC GN Panel,

inconclusive results, epidemiologic testing, recovery of organisms present in positive blood cultures samples, and susceptibility testing of bacteria in polymicrobial samples.

Testing is indicated for *Acinetobacter* spp., Enterobacterales, and *Pseudomonas aeruginosa*, as recognized by the FDA Susceptibility Test Interpretive Criteria (STIC).The PhAST BC GN Panel with the PhAST instrument has demonstrated acceptable performance with the following organisms:

Amikacin: Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Serratia marcescens*)

Ampicillin/sulbactam: *Acinetobacter* spp. (*Acinetobacter baumannii* complex)

Aztreonam: Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus vulgaris* group)

Cefepime: Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens*) and *Pseudomonas aeruginosa*

Ceftazidime: *Acinetobacter* spp. (*Acinetobacter baumannii* complex)*,* Enterobacterales (*Enterobacter cloacae* complex*, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens*) and *Pseudomonas aeruginosa*

Ceftazidime/avibactam: Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris* group*, Serratia marcescens*) and *Pseudomonas aeruginosa*

Ceftriaxone: Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis*)

Ciprofloxacin: Enterobacterales (*Citrobacter freundii* complex*, Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens*) and *Pseudomonas aeruginosa*

Ertapenem: Enterobacterales (*Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis*)

Gentamicin: Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris* group*, Serratia marcescens*)

Levofloxacin: Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca,*

Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris group*, Serratia marcescens*) and *Pseudomonas aeruginosa*

Meropenem: *Acinetobacter* spp. (*Acinetobacter baumannii* complex)*,* Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis*) and *Pseudomonas aeruginosa*

Piperacillin/tazobactam: *Acinetobacter* spp. (*Acinetobacter baumannii* complex)*,* Enterobacterales (*Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae*) and *Pseudomonas aeruginosa*

Tobramycin: Enterobacterales (*Citrobacter freundii* complex*, Citrobacter koseri, Enterobacter cloacae* complex*, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Proteus vulgaris* group) and *Pseudomonas aeruginosa*

Trimethoprim/sulfamethoxazole: Enterobacterales (*Citrobacter koseri, Escherichia coli, Klebsiella aerogenes, Klebsiella pneumoniae, Serratia marcescens*)

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

- The PhAST BC GN Panel should not be used for samples with Gram stain results that indicate the positive blood culture contains yeast or gram-positive bacteria or includes more than one organism (i.e., polymicrobial samples).
- The PhAST BC GN Panel does not replace the need to subculture the positive blood culture. A small percentage of polymicrobial samples may escape detection during Gram stain and/or the rapid ID method. The positive blood culture sample should be subcultured on a solid agar plate to confirm that the sample is monomicrobial. If the subcultured agar plate indicates that the sample is polymicrobial, the PhAST BC GN Panel results should be discarded. Susceptibility testing by alternate methods should be performed on each isolate type from the subcultured plate.
- The performance of this test has only been evaluated using the following blood culture bottles:
    - BD BACTEC Standard/10 Aerobic/F
    - BD BACTEC Standard/10 Anaerobic/F
    - BD BACTEC PEDS PLUS/F
    - BD BACTEC Plus Aerobic/F
    - BD BACTEC Plus Anaerobic/F
    - BD BACTEC Lytic Anaerobic/F
    - bioMerieux BacT/ALERT SA Standard Aerobic
    - bioMerieux BacT/ALERT SN Standard Anaerobic
    - bioMerieux BacT/ALERT FA Plus Aerobic
    - bioMerieux BacT/ALERT FN Plus Anaerobic
    - bioMerieux BacT/ALERT PF Plus
- Positive blood cultures should be tested immediately after a positive flag, where possible. In the case of unavoidable delays or if the need for re-testing arises, testing on the PhAST

BC GN Panel may be performed on positive blood culture bottles removed from the continuous monitoring system within 5 hours of bottle ring and subsequently kept at room temperature for up to 12 hours after removal.

- Positive blood culture samples must be loaded on the PhAST BC GN Panel cartridge and the run must be initiated within 10 minutes of lysing the positive blood culture sample.

The following limitations were added to the device labeling based on performance demonstrated in the current submission:

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

- *Amikacin: P. mirabilis, P. vulgaris group*
- *Ampicillin/sulbactam: Enterobacterales*
- *Aztreonam: P. mirabilis, S. marcescens, P. aeruginosa*
- *Cefepime: E. cloacae complex, P. vulgaris group*
- *Ceftazidime: C. freundii complex, C. koseri, E. coli, P. vulgaris group*
- *Ceftriaxone: P. vulgaris group, S. marcescens*
- *Ciprofloxacin: C. koseri, P. vulgaris group*
- *Ertapenem: C. freundii complex, C. koseri, E. cloacae complex, P. vulgaris group, S. marcescens,*
- *Meropenem: E. cloacae complex, P. vulgaris group, S. marcescens*
- *Piperacillin/tazobactam: C. freundii complex, C. koseri, P. mirabilis, P. vulgaris, S. marcescens*
- *Tobramycin: K. pneumoniae, P. mirabilis, S. marcescens*
- *Trimethoprim/sulfamethoxazole: C. freundii complex, E. cloacae complex, K. oxytoca, P. mirabilis, P. vulgaris group*

The ability of the PhAST BC GN Panel to detect resistance in the following antimicrobial/organism combinations is unknown because an insufficient number of resistant isolates were available during the clinical study:

- *Amikacin: C. koseri, K. aerogenes, K. oxytoca*
- *Aztreonam: C. koseri, P. vulgaris group*
- *Cefepime: C. koseri*
- *Ceftazidime: P. mirabilis*
- *Ceftazidime/avibactam: C. freundii complex, C. koseri, K. aerogenes, P. mirabilis, P. vulgaris group,*
- *Ertapenem: P. mirabilis*
- *Gentamicin: C. koseri, P. vulgaris group, S. marcescens*
- *Levofloxacin: C. koseri, P. vulgaris group*
- *Meropenem: C. koseri, P. mirabilis*
- *Tobramycin: C. koseri, P. vulgaris group*
- *Trimethoprim/sulfamethoxazole: C. koseri*

D Special Instrument Requirements:

The PhAST BC GN Panel must be used with the PhAST instrument (PhAST instrument with the Phase 1 releases: cloud release dcs1.0.11 and instrument release v1.0.7.).

IV Device/System Characteristics:

A Device Description:

The PhAST BC GN Panel is an *in vitro* diagnostic assay for qualitative (breakpoint) determination of antimicrobial susceptibility testing (AST) of pathogenic gram-negative bacteria from positive blood cultures (BC) and is intended to be used with the PhAST instrument. The PhAST BC GN Panel is a phenotypic test that utilizes video microscopy and analysis of single-cell phenotypes to determine susceptibility based on categorical interpretation. The PhAST BC GN Panel is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system and reported as monomicrobial gram-negative specimens by Gram staining. The PhAST BC GN Panel does not provide organism identification. Organism identity, using an FDA-cleared method, must be entered into the PhAST instrument or PhAST web application before categorical interpretation (Susceptible, Susceptible-Dose Dependent, Intermediate, Resistant, S/SDD/I/R) of antimicrobial susceptibility is reported. The PhAST BC GN Panel contains 15 antimicrobials. The workflow from a direct positive blood culture specimen requires less than one minute of hands-on time. PhAST BC GN Panel cartridges are run on-demand utilizing one of four independent, random-access bays on the PhAST instrument.

B Principle of Operation:

The PhAST instrument runs the PhAST BC GN Panel. The PhAST BC GN Panel is run by loading a positive blood culture sample onto an assay-specific, single-use cartridge, which is then inserted into the instrument. Each cartridge is loaded with one patient sample. The PhAST BC GN Panel cartridge contains 15 antimicrobials, each at 2 to 5 concentrations, dried down across 45 imaging chambers. An additional eight (8) chambers are used for positive controls and contain no antimicrobials. The PhAST BC GN Panel uses video-microscopy to automatically image and quantify a range of phenotypes of bacteria in the imaging chambers on the cartridge and how these phenotypes change in response to the antimicrobials relative to the (antimicrobial-free) growth control chambers. Video-microscopy data is gathered at three (3) time points and sent to PhAST's cloud for analysis. A susceptibility report is generated after the user enters the organism identity into the PhAST instrument or PhAST web application. The organism identity is determined via an external source, such as an FDA-cleared rapid molecular identification method or MALDI-TOF. The PhAST instrument can run four (4) PhAST BC GN Panel cartridges simultaneously and with random access.

To determine the susceptibility of organisms to a given antimicrobial, the Diagnostic Algorithm within the Data Analysis Pipeline compares phenotypes of the organisms in the no-antimicrobial, positive-growth control to the same phenotypes of the organisms under antimicrobial treatment at the different antimicrobial concentrations on the cartridge. From this comparison, the Diagnostic Algorithm computes a resistance score, which is used to determine the categorical interpretation (S, SDD, I, or R). The resistance score is not reported to the user. For samples where there remains significant ambiguity in the categorical interpretation (i.e., the S/SDD/I/R probabilities are below a given threshold), a "No Call: Inconclusive" result is reported to minimize the possibility of the user obtaining incorrect results. Cases of no-growth or low-growth, for which a reliable diagnostic output is precluded, are reported as "Inadequate Growth"

(for the entire sample). Cases in which a technical error prevents the result from being provided for a given antimicrobial, for example due to a bubble in the image or a focus problem, are reported as “Technical Error” (for that antimicrobial). In this case, the user is instructed to run the assay again one time using a new cartridge and, if the problem occurs again, to test with an alternate method.

The specific phenotypes utilized by the Diagnostic Algorithm vary by antimicrobial and by organism and are drawn from a broad range of phenotypes related to growth, morphology, and motility that are quantified from the video microscopy data at the single-cell level.

C Instrument Description Information:

1. Instrument Name:
   PhAST instrument

2. Specimen (Positive Blood Culture) Identification:
   A specimen identification label is applied to the PhAST BC GN Panel cartridge in the designated accession number barcode area, and to initiate a run, the user enters the specimen identification into the PhAST instrument software via the provided external barcode scanner or by manually typing it using the touchscreen's onscreen keyboard.

3. Specimen Sampling and Handling:
   The operator follows a streamlined workflow guided by the PhAST instrument touchscreen interface for sample preparation from positive blood cultures. The sample is prepared from a gram-negative and monomicrobial positive blood culture using a sterile collection method capable of obtaining approximately 1 mL of blood culture. The operator sterilizes the rubber septum with an alcohol pad and collects 1 mL of positive blood culture using either a 3 mL syringe with 18-gauge needle or subculture needle into a sterile 1.5 mL microcentrifuge tube.

   A 60 µL aliquot of the positive blood culture is transferred to a lysis buffer tube containing red blood cell lysis buffer using a P200 micropipette. The tube is inverted 10 times to ensure proper mixing without vortexing, and visual inspection confirms adequate mixing. Using the same pipette with a new sterile tip, 60 µL of the culture-lysis buffer mixture is collected and dispensed into the PhAST BC GN Panel cartridge through the designated inlet. The cartridge inlet is sealed by folding over the attached blue cap. The operator scans the cartridge barcode and accession number barcode, removes the protective film from the cartridge underside, and loads the cartridge into an available bay on the PhAST instrument. The system automatically performs sample metering, dilution with distilled water, mixing with dried Mueller Hinton broth using a magnetic mixing ball, and routes the prepared sample through microchannels to 56 imaging chambers containing dried antimicrobials at various concentrations for automated antimicrobial susceptibility testing.

4. Calibration:
The PhAST instrument automatically performs comprehensive self-checks at start-up and every 24 hours, including camera exposure calibration, linear actuator homing, pressure sensor calibration, and continuous monitoring of pneumatic, optical, and electronic subsystems. At the start of each assay, the system conducts Assay Start quality control checks including cartridge positioning calibration and pressure sensor verification, and initiation of AST testing can proceed only if all system functions and quality control checks pass successfully.

5. Quality Control:
Quality control (QC) is performed by the operator using manufacturer-specified organisms (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumoniae* CDC AR-0097, *Klebsiella pneumoniae* IHMA 23-084741-01, *Enterobacter cloacae* CDC AR-0432, *Escherichia coli* CDC AR-0434, and *Escherichia coli* ATCC NCTC13353) appropriate for each antimicrobial agent. The QC AST reports providing "Pass" or "Fail" results based on expected categorical interpretations for each strain and antimicrobial combination.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Accelerate Pheno System, Accelerate PhenoTest BC

B Predicate 510(k) Number(s):
DEN160032

C Comparison with Predicate(s):

Device & Predicate Device(s):	K253316 (Device)	DEN160032 (Predicate)
Device Trade Name	PhAST instrument and PhAST Blood Culture Gram-negative Panel	Accelerate Pheno System, Accelerate PhenoTest BC Kit
General Device Characteristic Similarities		
Intended Use	The PhAST Blood Culture Gram-negative Panel (PhAST BC GN Panel) is an *in vitro* diagnostic assay for qualitative (breakpoint) determination of antimicrobial susceptibility testing (AST) of pathogenic gram-negative bacteria from positive blood cultures (BC) and is intended to be used with the PhAST instrument. The PhAST BC GN Panel is a phenotypic test that utilizes video microscopy and analysis of single-cell phenotypes to determine susceptibility based on categorical interpretation. The PhAST BC GN Panel does not provide organism	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

	identification. The PhAST BC GN Panel is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system and reported as monomicrobial gram-negative specimens by Gram staining. Organism identity must be entered into the PhAST instrument or PhAST web application before categorical interpretation (Susceptible, Susceptible-Dose Dependent, Intermediate, Resistant, S/SDD/I/R) of antimicrobial susceptibility is reported. The PhAST BC GN Panel contains 15 antimicrobials. The PhAST BC GN Panel is intended to aid in the diagnosis and treatment of individuals suspected of bloodstream infection by a healthcare provider. Results should not be used as the sole basis for patient management decisions. This test is performed by laboratory health professionals in a clinical diagnostic setting. Results may be used as an aid to clinicians in determining appropriate antimicrobial therapy. Test results from the PhAST BC GN Panel should be interpreted 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. Sub-culturing is necessary to support further testing for: bacteria and antimicrobials not on the PhAST BC GN Panel, inconclusive results, epidemiologic testing, recovery of organisms present in positive blood cultures samples, and susceptibility testing of bacteria in polymicrobial samples.	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 Type	Blood cultures signaled as positive by a continuous monitoring blood culture system.	Same
Inoculation Method	Automated	Same
Read Method	Automated	Same
Technology	Single-cell, time-lapse microscopy	Similar

General Device Characteristic Differences		
IVD Functions	AST	ID and AST
Indicated Antimicrobials	Amikacin Ampicillin/sulbactam Aztreonam Cefepime Ceftazidime Ceftazidime/avibactam Ceftriaxone Ciprofloxacin Ertapenem Gentamicin Levofloxacin Meropenem Piperacillin/tazobactam Tobramycin Trimethoprim/ sulfamethoxazole	Amikacin Ampicillin/sulbactam Aztreonam Cefepime Ceftazidime Ceftriaxone Ciprofloxacin Ertapenem Gentamicin Meropenem Piperacillin/tazobactam Tobramycin Additional Gram-positive antimicrobials are also included on the Accelerate PhenoTest BC kit: Ampicillin Ceftaroline Daptomycin Linezolid Vancomycin
Tested Organism	Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter* spp.	Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Enterococcus* spp., *Staphylococcus* spp.
Sample Preparation	Short (<1 min) pre-processing step for red blood cell lysis prior to sample loading followed by automated reading	Automated
Time to AST Result	Range 91–154 minutes depending on drug and organism tested	Approximately 7 hours
AST Results	Report results as categorical interpretation	Report results as minimum inhibitory concentration (MIC) with categorical interpretation

In addition to the similarities and differences between the candidate and predicate device listed in the table above, the candidate device has an authorized PCCP to support the removal of specific labeling limitations solely related to insufficient resistant isolate representation when additional resistant isolates become available. The plan outlines the specific procedures and acceptance criteria that PhAST Corp. intends to use to evaluate whether labeling limitations for specific antimicrobial/organism combinations on the PhAST BC GN Panel can be removed based on supplemental resistant isolate data. The PCCP requires a minimum of 5 resistant isolates by reference method with no observed very major errors among the supporting resistant isolates, and continued compliance with the AST Special Controls Guidance criteria including categorical agreement >89.9% and error rates (i.e., very major error rate ≤2%, major error rate ≤3%) for each combination seeking limitation removal. PhAST Corp. will update the PhAST BC GN Panel labeling to remove the specific limitation after validation confirms that performance criteria continue to be met.

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 36, Performance Standards for Antimicrobial Susceptibility Testing; 2025.
- CLSI EP07 12th Edition, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 2024.
- CLSI EP37 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry.
- IEC 61326-2-6 Edition 3.0 2020-10 19-43, Electrical equipment for measurement control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment.
- IEC 61326-1 Edition 3.0 2020-10 19-42, Electrical equipment for measurement control and laboratory use - EMC requirements - Part 1: General requirements.
- IEC 61010-1 Edition 3.1 2017-01 CONSOLIDATED VERSION 19-34, Safety requirements for electrical equipment for measurement control and laboratory use - Part 1: General requirements [Including: Corrigendum 1 (2019)].
- ANSI AAMI IEC 62304:2006/A1:2016 13-79, Medical device software – Software life cycle processes [Including Amendment 1 (2016)].
- ANSI AAMI IEC 62366-1 Edition 1.1 2020-06 CONSOLIDATED VERSION, 5-129, Medical devices – Part 1: Application of usability engineering to medical devices.
- ANSI AAMI ISO 14971 Third Edition 2019-12 5-125, Medical devices – Application of risk management to medical devices.
- ANSI AAMI IEC TIR800002-1:2009, 13-34, Medical device software - Part 1: Guidance on the application of ISO 14971 to medical device software.
- ANSI ISA 62443-4-1-2018, 13-119, Security for industrial automation and control systems Part 4-1: Product security development life-cycle requirements.
- IEC 81001-5-1 Edition 1.0 2021-12, 13-122, Health software and health IT systems safety effectiveness and security - Part 5-1: Security - Activities in the product life cycle.
- UL ANSI 2900-1 1st Edition 2017, 13-96, Standard for Safety Standard for Software Cybersecurity Network-Connectable Products Part 1: General Requirements.
- AAMI TIR57:2016, 13-83, Principles for medical device security - Risk management.
- AAMI TIR97:2019, 13-112, Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.
- ISO 20417 1st edition 2021-04 Corrected version 2021-12, 5-135, Medical Devices – Information to be supplied by the manufacturer.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   A reproducibility study of the PhAST BC GN Panel with the PhAST instrument was conducted using contrived positive blood culture bottles prepared with sixteen (16) bacterial strains from *Acinetobacter* spp., Enterobacterales, and *P. aeruginosa* with at least one susceptible and one resistant strain within each organism reporting group. Triplicate samples from each contrived blood culture were tested at three sites over three consecutive days to assess reproducibility across instruments, locations, operators, and testing days (≥10 isolates × 3 sites × 3 replicates × 3 days = ≥270 results per antimicrobial). Reproducibility was determined for each organism/antimicrobial combination as the Categorical Agreement (CA) between each result and the categorical mode across all tests for that organism/antimicrobial combination. Both inter-site and intra-site reproducibility were evaluated for each antimicrobial on the PhAST BC GN Panel.

   The reproducibility results demonstrated acceptable CA performance of greater than 95% for each antimicrobial. A summary of the reproducibility results is provided in **Table 1**.

   Table 1. Summary of Reproducibility Results of the PhAST Blood Culture Gram-Negative Panel with the PhAST Instrument

Antimicrobial	No. isolates Tested	Total No. Tests	Categorical Agreement (CA)
Amikacin	11	309	97.1% (300/309)
Ampicillin/sulbactam	11	307	99.0% (304/307)
Aztreonam	11	339	100% (339/339)
Cefepime	13	393	99.7% (422/423)
Ceftazidime	16	475	99.6% (473/475)
Ceftazidime/avibactam	13	382	100% (382/382)
Ceftriaxone	11	333	100% (333/333)
Ciprofloxacin	13	394	100% (394/394)
Ertapenem	9	284	97.5% (277/284)
Gentamicin	11	335	100% (335/335)
Levofloxacin	13	396	99.7% (395/396)
Meropenem	16	467	99.8% (466/467)
Piperacillin/tazobactam	16	473	97.3% (460/473)
Tobramycin	13	382	98.7% (377/382)
Trimethoprim/sulfamethoxazole	11	336	100% (336/336)

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 PhAST BC GN Panel with the PhAST instrument and the ability of the PhAST instrument to prepare an appropriate inoculum regardless of the starting concentration. A panel of seventeen (17) bacterial strains including at least one susceptible and one resistant strain from each organism reporting group (i.e., Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp.) was tested at four target inoculum concentrations: 1, 10, 100, and 1000 CFU/mL. Each strain was spiked into whole human blood at each concentration and incubated in BacT/ALERT FA Plus Aerobic blood culture

bottles until flagged positive. Four biological replicates were prepared for each strain and inoculum concentration to meet the minimum target of 45 data points per antimicrobial and target concentration.

The organism concentration in each contrived sample was confirmed by colony count. PhAST BC GN Panel results were compared to modal reference broth microdilution (BMD) categorical interpretation results (S, SDD, I, or R). The PhAST BC GN Panel with the PhAST instrument demonstrated acceptable performance (**Tables 2**), with a CA of >90% for each antimicrobial and target inoculum concentrations.

Table 2. Categorical Agreement Results for each Target Inoculum Concentration

Antimicrobial	Categorical Agreement (%)			
	1 CFU/mL	10 CFU/mL	100 CFU/mL	1,000 CFU/mL
Amikacin	45/45 (100%)	46/46 (100%)	45/46 (97.8%)	46/47 (97.9%)
Ampicillin/sulbactam	44/48 (91.7%)	49/50 (98.0%)	51/51 (100%)	48/49 (98.0%)
Aztreonam	48/48 (100%)	48/48 (100%)	48/48 (100%)	47/47 (100%)
Cefepime	52/56 (92.9%)	49/53 (92.5%)	52/56 (92.9%)	51/55 (92.7%)
Ceftazidime	67/67 (100%)	63/64 (98.4%)	67/67 (100%)	67/67 (100%)
Ceftazidime/avibactam	56/56 (100%)	54/54 (100%)	55/55 (100%)	55/55 (100%)
Ceftriaxone	47/47 (100%)	48/48 (100%)	48/48 (100%)	46/46 (100%)
Ciprofloxacin	55/55 (100%)	52/52 (100%)	54/54 (100%)	54/54 (100%)
Ertapenem	46/46 (100%)	46/46 (100%)	46/46 (100%)	45/45 (100%)
Gentamicin	48/48 (100%)	47/47 (100%)	48/48 (100%)	46/46 (100%)
Levofloxacin	56/56 (100%)	53/54 (98.1%)	54/54 (100%)	54/54 (100%)
Meropenem	68/68 (100%)	63/63 (100%)	67/67 (100%)	63/64 (98.4%)
Piperacillin/tazobactam	65/66 (98.5%)	66/66 (100%)	65/68 (95.6%)	66/66 (100%)
Tobramycin	45/46 (97.8%)	46/47 (97.9%)	51/51 (100%)	48/50 (96.0%)
Trimethoprim/ sulfamethoxazole	48/48 (100%)	48/48 (100%)	47/47 (100%)	47/47 (100%)

3. Linearity:

Not applicable

4. Analytical Specificity/Interference:

Endogenous/Exogenous Interference Studies:
An interfering substances study was performed to evaluate if substances naturally present or artificially introduced into blood culture bottles affect the performance of the PhAST BC GN Panel with the PhAST instrument. A panel of seventeen (17) bacterial strains including at least one susceptible and one resistant strain from each organism reporting group (i.e., Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp.) was evaluated. The nine interfering substances were spiked into BacT/ALERT FA Plus Aerobic blood culture bottles at or above clinically relevant concentrations along with representative organisms and tested in triplicate. Control bottles were seeded with organism and no potential interferent. Bottles were incubated in a continuous monitoring blood culture system until positivity. As this was a method-to-method comparison determined by comparing categorical interpretation results (S,

SDD, I, or R) from interferent-containing samples with modal results from control samples, CA of ≥95% was deemed acceptable.

Overall, the presence of high concentrations of the tested interferents in blood cultures did not significantly interfere with PhAST BC GN Panel results, with most combinations achieving acceptable performance (CA ≥95%) (**Tables 3 and 4**). Specific antimicrobial/organism combinations that demonstrated CA <95% are discussed below.

Ertapenem, ampicillin/sulbactam, piperacillin/tazobactam, and tobramycin in the presence of triglycerides at 5 mg/mL had a CA <95% for Enterobacterales species. Repeat testing of triglycerides at 1.67 mg/mL demonstrated acceptable performance.

Tobramycin in the presence of conjugated bilirubin at 400 mg/L had a CA <95% for *P. aeruginosa*. Repeat testing of conjugated bilirubin at 133 mg/L demonstrated acceptable performance.

Footnotes in the performance tables (**Tables 3 and 4**) were included in device labeling to address the performance concerns of these interfering substances.

Table 3. Overall Performance with Potential Interfering Substances (Part 1)

Antimicrobial	Categorical Agreement (%)				
	Conjugated Bilirubin (≥400 mg/L)	Unconjugated Bilirubin (≥400 mg/L)	Gamma Globulin (≥50 g/L)	Triglycerides (≥5 mg/mL)	Red Blood Cells (≥20 g/dL)
Amikacin	41/41 (100%)	38/38 (100%)	38/38 (100%)	33/34 (97.1%)	37/37 (100%)
Ampicillin/sulbactam	36/36 (100%)	38/38 (100%)	38/38 (100%)	35/38 (92.1%)[a]	37/37 (100%)
Aztreonam	36/36 (100%)	38/38 (100%)	36/36 (100%)	36/36 (100%)	38/38 (100%)
Cefepime	42/42 (100%)	38/38 (100%)	39/39 (100%)	42/42 (100%)	44/44 (100%)
Ceftazidime	50/50 (100%)	47/47 (100%)	48/48 (100%)	49/50 (98.0%)	50/50 (100%)
Ceftazidime/avibactam	42/42 (100%)	38/38 (100%)	39/39 (100%)	42/42 (100%)	44/44 (100%)
Ceftriaxone	36/36 (100%)	38/38 (100%)	36/36 (100%)	36/36 (100%)	38/38 (100%)
Ciprofloxacin	42/42 (100%)	38/38 (100%)	39/39 (100%)	40/40 (100%)	41/41 (100%)
Ertapenem	37/37 (100%)	37/37 (100%)	38/38 (100%)	34/36 (94.4%)[a]	38/38 (100%)
Gentamicin	41/41 (100%)	36/37 (97.3%)	35/36 (97.2%)	37/37 (100%)	38/38 (100%)
Levofloxacin	40/41 (97.6%)	36/37 (97.3%)	37/37 (100%)	42/42 (100%)	41/41 (100%)
Meropenem	50/50 (100%)	44/44 (100%)	48/48 (100%)	49/49 (100%)	49/50 (98.0%)
Piperacillin/tazobactam	48/50 (96.0%)	46/46 (100%)	47/47 (100%)	42/46 (91.3%)[a]	48/49 (98.0%)

Tobramycin	36/39 (92.3%)[b]	40/41 (97.6%)	42/42 (100%)	33/35 (94.3%)[a]	40/40 (100%)
Trimethoprim/ sulfamethoxazole	41/41 (100%)	38/38 (100%)	36/36 (100%)	36/36 (100%)	38/38 (100%)

[a] Ertapenem, ampicillin/sulbactam, tobramycin and piperacillin/tazobactam demonstrated <95% Categorical Agreement (CA) at the tested concentration due to Enterobacterales isolates. When retested with 1.67 mg/mL triglycerides, performance was acceptable.
[b] Tobramycin demonstrated <95% CA at the tested concentration due to one *P. aeruginosa* isolate. When retested with 133 mg/L conjugated bilirubin, performance was acceptable.

Table 4. Overall Performance with Potential Interfering Substances (Part 2)

Antimicrobial	Categorical Agreement (%)			
	White Blood Cells (≥12,000 WBC/µL)	Platelets (≥400,000/µL)	Heparin (≥3000 Units/L)	SPS (≥0.1% w/v)
Amikacin	37/37 (100%)	36/36 (100%)	41/41 (100%)	36/36 (100%)
Ampicillin/sulbactam	48/49 (98.0%)	48/49 (98.0%)	51/51 (100%)	45/45 (100%)
Aztreonam	36/36 (100%)	36/36 (100%)	39/39 (100%)	38/39 (97.4%)
Cefepime	42/42 (100%)	42/42 (100%)	42/42 (100%)	39/39 (100%)
Ceftazidime	42/42 (100%)	35/36 (97.2%)	48/48 (100%)	47/47 (100%)
Ceftazidime/avibactam	42/42 (100%)	42/42 (100%)	42/42 (100%)	39/39 (100%)
Ceftriaxone	50/50 (100%)	51/51 (100%)	60/60 (100%)	48/48 (100%)
Ciprofloxacin	42/42 (100%)	42/42 (100%)	42/42 (100%)	39/39 (100%)
Ertapenem	38/38 (100%)	36/36 (100%)	41/41 (100%)	36/36 (100%)
Gentamicin	36/36 (100%)	36/36 (100%)	36/36 (100%)	40/40 (100%)
Levofloxacin	38/38 (100%)	36/36 (100%)	41/41 (100%)	36/36 (100%)
Meropenem	41/42 (97.6%)	42/42 (100%)	41/42 (97.6%)	39/39 (100%)
Piperacillin/tazobactam	38/38 (100%)	36/36 (100%)	41/41 (100%)	36/36 (100%)
Tobramycin	39/39 (100%)	40/40 (100%)	38/40 (95.0%)	36/36 (100%)
Trimethoprim/ sulfamethoxazole	51/51 (100%)	48/49 (98.0%)	51/51 (100%)	48/48 (100%)

Antimicrobial Interference Studies:
A study was performed to evaluate if antimicrobials present in blood culture bottles affect the performance of PhAST BC GN Panel with the PhAST instrument using seeded positive blood culture samples with and without interfering antimicrobials. Four representative antimicrobials from different drug classes were tested including ciprofloxacin (fluoroquinolones), ceftriaxone (cephalosporins), ampicillin/sulbactam (β-lactam/β-lactamase

inhibitor), and amikacin (aminoglycosides). A panel of ten (10) bacterial strains including at least one resistant strain from each organism reporting group (i.e., Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp.) was tested for each interfering antimicrobial. Antimicrobial interferents were spiked into resin-free BacT/ALERT SA Standard Aerobic blood culture bottles at the highest drug concentrations as recommended by CLSI EP37. Control bottles were seeded with organisms and no antimicrobial interferent. As this was a method-to-method comparison determined by comparing categorical interpretation results (S, SDD, I, or R) from interferent-containing samples with modal results from control samples, CA of ≥95% was deemed acceptable.

Overall, the presence of high concentrations of the tested interferents in blood cultures does not significantly interfere with the results of the PhAST BC GN Panel, with most combinations achieving acceptable performance (CA ≥95%) (**Table 5**). Specific antimicrobial/organism combinations that demonstrated CA <95% are discussed below.

Ceftazidime/avibactam in the presence of amikacin had a CA <95% due to a single very major error with one *E. cloacae* isolate, which was determined to be a technical error (i.e., focus problem) rather than antimicrobial interference.

Piperacillin/tazobactam in the presence of ceftriaxone had a CA <95% due to a single minor error with one replicate of a single *K. pneumoniae* isolate.

Tobramycin in the presence of ciprofloxacin had a CA <95% due to minor errors with one *P. aeruginosa* isolate and one *K. pneumoniae* isolate. Supplemental testing of two additional *P. aeruginosa* isolates as well as one *K. pneumoniae* isolate in the presence of ciprofloxacin at three-fold lower concentration (0.13 mg/dL) demonstrated acceptable performance.

Tobramycin in the presence of amikacin had a CA <95% due to three (3) minor errors with one *P. aeruginosa* isolate. Supplemental testing of two additional *P. aeruginosa* isolates demonstrated acceptable performance.

Amikacin in the presence of ciprofloxacin (0.4 mg/dL) and ampicillin/sulbactam (2.5/1.25 mg/dL) had a CA <95% due to one *K. oxytoca* isolate. Repeat testing with three-fold lower concentrations of ciprofloxacin (0.13 mg/dL) or ampicillin/sulbactam (0.83/0.42 mg/dL), demonstrated <95% CA due to minor errors of the same *K. oxytoca* isolate.

Footnotes in the performance table (**Table 5**) were included in device labeling to address the performance concerns of these specific interferences.

Table 5. Overall Performance with Potential Interfering Antimicrobials.

Antimicrobial	Categorical Agreement (%)			
	Ciprofloxacin (0.4 mg/dL)	**Ceftriaxone (28 mg/dL)**	**Ampicillin/ sulbactam (2.5/1.25 mg/dL)**	**Amikacin (4.8 mg/dL)**
Amikacin	16/18 (88.9%)[a]	15/15 (100%)	15/18 (83.3%)[a]	15/15 (100%)
Ampicillin/sulbactam	17/17 (100%)	16/16 (100%)	20/20 (100%)	15/15 (100%)

Aztreonam	20/20 (100%)	19/19 (100%)	19/19 (100%)	15/15 (100%)
Cefepime	25/25 (100%)	19/19 (100%)	19/19 (100%)	20/20 (100%)
Ceftazidime	25/25 (100%)	19/19 (100%)	25/25 (100%)	30/30 (100%)
Ceftazidime/avibactam	24/24 (100%)	18/18 (100%)	18/18 (100%)	14/15 (93.3%)[b]
Ceftriaxone	20/20 (100%)	18/18 (100%)	20/20 (100%)	17/17 (100%)
Ciprofloxacin	25/25 (100%)	19/19 (100%)	18/18 (100%)	20/20 (100%)
Ertapenem	20/20 (100%)	19/19 (100%)	19/19 (100%)	17/17 (100%)
Gentamicin	20/20 (100%)	19/19 (100%)	20/20 (100%)	15/15 (100%)
Levofloxacin	24/25 (96.0%)	19/19 (100%)	20/20 (100%)	20/20 (100%)
Meropenem	24/24 (100%)	18/18 (100%)	25/25 (100%)	27/28 (96.4%)
Piperacillin/tazobactam	24/24 (100%)	18/19 (94.7%)[c]	25/25 (100%)	28/28 (100%)
Tobramycin	16/22 (72.7%)[d]	14/14 (100%)	18/18 (100%)	17/20 (85.0%)[e]
Trimethoprim/ sulfamethoxazole	20/20 (100%)	19/19 (100%)	20/20 (100%)	15/15 (100%)

[a] Amikacin in the presence of ciprofloxacin (0.4 mg/dL) or ampicillin/sulbactam (2.5/1.25 mg/dL) had a CA <95% due to one *K. oxytoca* strain tested. Repeat testing with three-fold lower concentrations of ciprofloxacin (0.13 mg/dL) or ampicillin/sulbactam (0.83/0.42 mg/dL), demonstrated CA <95% due to minor errors of the same *K. oxytoca* strain.

[b] Ceftazidime/avibactam in the presence of amikacin had a CA <95% due to a single very major error, which was determined to be a technical error.

[c] Piperacillin/tazobactam in the presence of ceftriaxone had a CA <95% due to a single minor error with one replicate of a single *K. pneumonia* isolate.

[d] Tobramycin in the presence of ciprofloxacin (0.4 mg/dL) had a CA <95% due to minor errors with one *P. aeruginosa* isolate and one *K. pneumoniae* isolate. Supplemental testing of two additional *P. aeruginosa* isolates as well as one *K. pneumoniae* isolate at three-fold lower ciprofloxacin concentration (0.13 mg/dL) demonstrated acceptable performance.

[e] Tobramycin in the presence of amikacin had a CA <95% due to three minor errors with one *P. aeruginosa* isolate. Supplemental testing of two additional *P. aeruginosa* isolates demonstrated acceptable performance.

5. Media Equivalency Study:

The PhAST BC GN Panel with the PhAST instrument was tested for compatibility with eleven different blood culture bottles from two manufacturers (BD BACTEC and bioMérieux BacT/ALERT). A panel of seventeen (17) bacterial strains including at least one susceptible and one resistant strain from each organism reporting group (i.e., Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp.) were individually mixed with human whole blood, seeded directly into blood culture bottles, and incubated for growth in continuous monitoring blood culture systems positivity. At least five (5) replicates of each resulting positive blood culture were tested using the PhAST BC GN Panel and PhAST instrument. Strict aerobic species (*P. aeruginosa* and *Acinetobacter* spp.) were not tested in anaerobic bottles.

PhAST BC GN Panel results of each bottle type with each antimicrobial/organism combination were compared to the modal reference broth microdilution (BMD) categorical interpretation results (S, SDD, I, or R). PhAST BC GN Panel results were also combined and analyzed based on blood culture bottle function and composition (i.e., aerobic with resin, aerobic without resin, pediatric aerobic with resin, anaerobic with resin, anaerobic without resin, and lytic anaerobic). The number of tests for each aggregated bottle type for each antimicrobial ranged from 51 to 82. Results for all antimicrobial/organism combinations demonstrated acceptable performance (≥90% CA) for all bottle types.

6. Detection Limit and Assay Reportable Range:

   Not applicable

7. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
   Quality control (QC) testing was performed each day that testing was conducted. The QC panel consists of seven (7) CLSI recommended or independently validated QC strains, which included strains to provide one susceptible (S) and one resistant (R) QC result for each of the 15 antimicrobials on the panel. QC strains for each antimicrobial were tested a sufficient number of times (i.e., at least 20 times/site) at each testing site using the PhAST BC GN Panel with the PhAST instrument, including the reference site using the broth microdilution reference method. QC organisms were tested on a rotating basis.

   QC expected ranges and results for the PhAST BC GN Panel are summarized in **Table 6**. For all antimicrobials, greater than 95% of results obtained during the clinical study were within the expected categorical interpretation (i.e., susceptible or resistant), which is acceptable.

Table 6. QC Results for the PhAST Blood Culture Gram-Negative Panel

Antimicrobial	QC Strains	Expected PhAST BC GN Panel Categorical Interpretation	PhAST BC GN Panel Results in Agreement (%)	Expected CLSI/Validated Reference MIC Range (µg/mL)	Reference MIC Results In Range (%)
Amikacin	*E. coli* ATCC 25922	S	93/94 (98.9%)	0.5 - 4	142/142 (100%)
	K. pneumoniae CDC AR-0097[a]	R	92/94 (97.9%)	128 - 512	142/142 (100%)
Ampicillin/ sulbactam	*E. coli* ATCC 25922	S	93/95 (97.9%)	2/1 - 8/4	142/142 (100%)
	K. pneumoniae IHMA 23-084731-01[a]	R	93/93 (100%)	64 - 256	150/150 (100%)
Aztreonam	*E. coli* ATCC 25922	S	93/93 (100%)	0.06 - 0.5	142/142 (100%)
	E. coli NCTC 13353[a]	R	93/94 (98.9%)	128 - 512	146/146 (100%)
Cefepime	*K. pneumoniae* ATCC 700603	S	93/93 (100%)	0.5 - 2	144/144 (100%)
	E. coli NCTC 13353[a]	R	93/94 (98.9%)	≥64	146/146 (100%)
Ceftazidime	*E. coli* ATCC 25922	S	93/93 (100%)	<0.12 - 0.5	142/142 (100%)
	E. coli NCTC 13353[a]	R	93/94 (98.9%)	32 - 256	146/146 (100%)
Ceftazidime/ avibactam	*E. coli* NCTC 13353[a]	S	93/93 (100%)	0.12 - 0.5	146/146 (100%)
	E. coli CDC AR-0434[a]	R	91/93 (97.8%)	32 - 128	152/152 (100%)
Ceftriaxone	*E. coli* ATCC 25922	S	93/93 (100%)	0.03 - 0.12	142/142 (100%)
	K. pneumoniae ATCC 700603[a]	R	93/93 (100%)	2 - 16	144/144 (100%)
Ciprofloxacin	*E. cloacae* CDC AR-0432[a]	S	93/93 (100%)	0.03 - 0.12	144/144 (100%)
	E. coli NCTC 13353[a]	R	93/93 (100%)	8 - 32	146/146 (100%)
Ertapenem	*E. coli* NCTC 13353[a]	S	93/93 (100%)	0.06 - 0.5	146/146 (100%)

	K. pneumoniae IHMA 23-084731-01[a]	R	93/94 (98.9%)	2 - 8	150/150 (100%)
Gentamicin	*E. cloacae* CDC AR-0432[a]	S	93/95 (97.9%)	0.25 - 1	144/144 (100%)
	E. coli NCTC 13353[a]	R	93/94 (98.9%)	128 - 512	146/146 (100%)
Levofloxacin	*E. cloacae* CDC AR-0432[a]	S	93/93 (100.0%)	0.03 - 0.125	144/144 (100%)
	K. pneumoniae CDC AR-0097[a]	R	93/94 (98.9%)	32 - 128	142/142 (100%)
Meropenem	*K. pneumoniae* IHMA 23-084731-01[a]	S	92/92 (96.8%)	0.06 - 0.5	144/150 (96.0%)
	K. pneumoniae CDC AR-0097[a]	R	93/94 (98.9%)	32 - 128	142/142 (100%)
Piperacillin/ tazobactam	*E. coli* ATCC 25922	S	93/93 (100%)	1 - 8	142/142 (100%)
	K. pneumoniae IHMA 23-084731-01[a]	R	93/93 (100%)	128 - 512	150/150 (100%)
Tobramycin	*E. cloacae* CDC AR-0432[a]	S	93/93 (100%)	0.25 - 1	144/144 (100%)
	K. pneumoniae CDC AR-0097[a]	R	93/93 (100%)	32 - 128	142/142 (100%)
Trimethoprim/ sulfamethoxazole	*E. cloacae* CDC AR-0432[a]	S	93/93 (100%)	0.06 - 0.25	144/144 (100%)
	E. coli CDC AR-0434[a]	R	92/92 (100%)	16 - 64	152/152 (100%)

[a] Validation performed for non-CLSI recommended QC strain

Validation of New QC Strains

To validate the use of seven (7) QC strains (i.e., *E. coli* ATCC 25922, *K. pneumoniae* CDC AR-0097, *K. pneumoniae* IHMA 23-084731-01, *E. coli* NCTC 13353, *K. pneumoniae* ATCC 700603, *E. coli* CDC AR-0434, and *E. cloacae* CDC AR-0432) with the PhAST BC GN Panel and PhAST instrument, the BMD reference method was performed to establish the expected BMD MIC range. Testing was performed with at least 80 replicates and two reagent lots over two days. For all antimicrobials, >95% of results were within the newly established MIC reference ranges, which is acceptable. The QC strains were then tested with the PhAST BC GN Panel using at least 80 replicates. For all antimicrobials, >95% of results were in agreement with the expected categorical interpretation, which is acceptable. Taken together, the acceptable performance supports the use of the seven QC strains with the PhAST BC GN Panel and PhAST instrument.

Purity Check

Purity plates were prepared from the positive blood cultures of every sample tested. AST results were only reported for pure isolates; data generated from plates that generated multiple colony morphologies was excluded from analyses.

Device Failure

Several types of device or test failures were observed and accounted for in the study:

- Assay Start QC Failures: Twenty tests did not initiate due to an automated quality check failure. The system detected these issues, and all 20 samples were successfully re-tested with a new cartridge, yielding valid results.
- User-Cancelled Tests: Two tests were cancelled by the user at external clinical sites.
- The combined “Technical Error” and “No Call: Inconclusive” result rates for each antimicrobial/organism group combination were assessed during the clinical study and was ≤10% unless otherwise noted.

Growth Failure Rate
There were 17 "Inadequate Growth" results from the total samples tested (1.9%). These full sample results were not included in the performance analysis.

8. Sample Stability Study

The purpose of this study was to demonstrate that positive blood culture samples that remain in the continuous monitoring system for up to 5 hours after bottle ring and/or held at room temperature for up to 12 hours after removal from the continuous monitoring system could provide accurate categorical interpretation results using the PhAST GN BC Panel with the PhAST instrument.

Contrived positive blood culture specimens (prepared in BacT/ALERT FA Plus Aerobic blood culture bottles) containing the recommended blood volume were tested across three storage conditions: immediate testing (T0), extended incubator storage (14 hours at 35°C), and mixed storage simulating clinical workflow delays (5 hours at 35°C followed by 14 hours at room temperature). A panel of sixteen (16) bacterial strains including at least one susceptible and one resistant strain from each organism reporting group (i.e., Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp.) was evaluated under three different storage conditions for each of the 15 antimicrobial agents on the panel. Each strain was tested in triplicate across all conditions. Categorical interpretation results (S, SDD, I, or R) at different storage timepoints were compared to the modal PhAST BC GN Panel results determined at T0. As this was a method-to-method comparison, CA ≥95% for each antimicrobial agent was considered acceptable.

The study demonstrated acceptable performance of ≥95% CA for both extended storage conditions (i.e., 14 hours at 35°C, and 5 hours at 35°C followed by 14 hours at room temperature) for each antimicrobial when compared to results obtained at T0 (**Table 7**).

As noted in the instructions for use, positive blood cultures should be tested immediately after a positive flag, where possible. In the case of unavoidable delays or if the need for re-testing arises, testing on the PhAST BC GN Panel may be performed on positive blood culture bottles removed from the continuous monitoring system within 5 hours of bottle ring and subsequently kept at room temperature for up to 12 hours after removal.

Table 7. Summary of Sample Stability Results of the PhAST Blood Culture Gram-Negative Panel with the PhAST Instrument

Antimicrobial	Categorical Agreement (%)	
	14h 35ºC vs. T0	5h 35ºC + 14h RT vs. T0
Amikacin	31/31 (100%)	32/32 (100%)
Ampicillin/sulbactam	28/29 (96.6%)	30/30 (100%)
Aztreonam	31/31 (100%)	32/32 (100%)
Cefepime	37/37 (100%)	37/37 (100%)
Ceftazidime	46/46 (100%)	47/47 (100%)
Ceftazidime/avibactam	37/37 (100%)	38/38 (100%)
Ceftriaxone	31/31 (100%)	31/31 (100%)
Ciprofloxacin	36/36 (100%)	38/38 (100%)
Ertapenem	33/34 (97.1%)	34/35 (97.1%)
Gentamicin	31/31 (100%)	32/32 (100%)
Levofloxacin	37/37 (100%)	38/38 (100%)

Meropenem	46/46 (100%)	46/46 (100%)
Piperacillin/tazobactam	45/45 (100%)	46/46 (100%)
Tobramycin	32/32 (100%)	35/35 (100%)
Trimethoprim/sulfamethoxazole	31/31 (100%)	31/31 (100%)

9. Assay Cut-Off:

   Not applicable

10. Cross Contamination/Carry-Over:
    The Carryover/Cross-Contamination Study was designed to evaluate the possibility of sample carryover on the PhAST instrument with the PhAST BC GN Panel across samples run at different times within the same bay or cross-contamination between the instrument's four bays. Contrived blood culture samples seeded with susceptible or resistant isolates were tested with the PhAST BC GN Panel in alternating patterns to detect carryover or cross-contamination. Testing was performed across 5 runs and 4 bays in a predetermined alternating pattern designed to detect both cross-contamination between bays within a single run and carryover on a single bay across multiple runs. All tested antimicrobials achieved 100% categorical agreement compared to reference BMD results, which were acceptable.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Clinical performance testing using the PhAST BC GN Panel with PhAST instrument was conducted at four sites (three geographically diverse US clinical sites and one internal site). For instances in which testing was required to supplement existing data from the original study and support specific claims, testing was performed using the PhAST BC GN Panel with the PhAST instrument at one internal site. Performance was evaluated using prospectively collected, fresh leftover positive blood culture samples from patients with suspected bacteremia. Samples were confirmed by Gram stain to contain only gram-negative bacteria prior to testing on the PhAST BC GN Panel. Additionally, blood culture bottles containing fresh human donor blood were contrived with clinical stock isolates and challenge isolates to supplement the fresh prospective blood cultures for lower prevalence species and resistance profiles. Subcultures of all positive blood cultures onto appropriate media (Tryptic Soy Agar with 5% sheep blood) were used to check for purity prior to preparing a frozen stock for shipment to the central reference laboratory. Polymicrobial samples were excluded from analysis. Organism identification was obtained from an FDA-cleared MALDI-TOF method for input into the PhAST web application to generate antimicrobial susceptibility test (AST) results.

   A total of 1028 samples were enrolled in the study, of which 890 samples were included in the performance analysis (256 fresh prospective positive blood culture samples, 121 samples contrived with clinical stock isolates, and 513 samples contrived with challenge isolates). In total, 138 samples were excluded from final performance analyses due to not meeting inclusion criteria, invalid QC results, or other protocol deviations.

   Performance was determined by comparing the categorical interpretation results (S, SDD, I, or R) of the PhAST BC GN Panel to the modal categorical interpretation results based on the minimum inhibitory concentration (MIC) values obtained from the reference broth

microdilution (BMD) method as described in the CLSI document *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, M07, 12th edition*. Performance was generally based on criteria outlined in the Class II Special Controls Document: Antimicrobial Susceptibility Test (AST) Systems including categorical agreement (CA) and categorical errors (minor, major, and very major errors) for each antimicrobial agent. CA was calculated as the percentage of PhAST BC GN Panel categorical interpretive results (S, SDD, I, or R) that were identical to the interpretive categories of the reference BMD result.

A summary of the performance of the PhAST BC GN Panel is described below for each antimicrobial agent with indicated species. Complete details including CA and error rate analysis per organism group are summarized in **Table 8**.

The time to result (TTR) of the PhAST BC GN Panel with the PhAST instrument was assessed across samples tested during the clinical study. The TTR was measured from the time the cartridge was loaded into the instrument to the generation of the susceptibility report following organism identity entry. The observed mean TTR was as follows:

- Enterobacterales: 99 ± 7 minutes (mean ± standard deviation), ranged from 91 minutes to 130 minutes.
- *Acinetobacter* spp.: 99 ± 7 minutes (mean ± standard deviation), ranged from 91 minutes to 116 minutes.
- *Pseudomonas aeruginosa*: 141 ± 6 minutes (mean ± standard deviation), ranged from 135 minutes to 154 minutes.

Amikacin/Enterobacterales. A total of 508 Enterobacterales (27 *C. freundii* complex, 37 *C. koseri*, 39 *E. cloacae* complex, 164 *E. coli,* 40 *K. aerogenes,* 37 *K. oxytoca,* 81 *K. pneumoniae*, 36 *P. mirabilis*, 9 *P. vulgaris* group, and 38 *S. marcescens*) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 95.2%, which was acceptable. There were 20 minor errors, 6 major errors (6/457 = 1.3%), and 1 very major error (1/34 = 2.9%).

When evaluating results by individual species, *P. mirabilis* demonstrated a CA of 86.1%, which is not acceptable. *P. mirabilis* had one major error (1/3 = 33.3%), which was considered a random error due to the limited number of resistant isolates tested. *P. vulgaris* group demonstrated a CA of 88.9%, which is not acceptable. Due to the unacceptable CA, these antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
  - *Amikacin: P. mirabilis, P. vulgaris group*

A limitation is included in the device labeling to address the lack of testing with resistant *Citrobacter koseri, K. aerogenes* and *K. oxytoca* isolates.

Ampicillin/sulbactam/Enterobacterales. A total of 332 Enterobacterales (19 *C. koseri*, 162 *E. coli,* 25 *K. oxytoca,* 76 *K. pneumoniae*, 36 *P. mirabilis*, and 14 *P. vulgaris* group) were

evaluated. The combined results from clinical and challenge testing demonstrated a CA of 58.7%, which is not acceptable. There were 69 minor errors, 68 major errors (68/251 = 27.1%), and no very major errors. Due to the unacceptable CA and major error rate, this antimicrobial/organism group combination is not indicated for use with the PhAST BC GN Panel, and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combination(s):*
  - *Ampicillin/sulbactam: Enterobacterales*

Ampicillin/sulbactam/*Acinetobacter* spp. A total of 62 *A. baumannii* complex were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 91.9%. There were 4 minor errors, 1 major error (1/35 = 2.9%), and no very major errors. Overall performance is acceptable.

Aztreonam/Enterobacterales. A total of 494 Enterobacterales isolates (25 *C. freundii* complex, 18 *C. koseri*, 43 *E. cloacae* complex, 170 *E. coli*, 31 *K. aerogenes*, 26 *K. oxytoca*, 82 *K. pneumoniae*, 14 *P. vulgaris* group, 44 *P. mirabilis*, and 41 *S. marcescens*) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 96.6%. There were 14 minor errors, 3 major errors (3/357 = 0.8%), and no very major errors. Overall performance is acceptable.

When evaluating results by individual species, *S. marcescens* had 2 major errors (2/35 = 5.7%), which is not acceptable. A limitation is included in the device labeling to address the unacceptable major error rate. In addition, *P. mirabilis* met all acceptance criteria in the clinical study; however, based on other results from supplemental analytical testing, this antimicrobial/organism combination was removed from the Indications for Use. These antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel, and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
  - *Aztreonam: P. mirabilis, S. marcescens*

A limitation is included in the device labeling to address the lack of testing with resistant *C. koseri* and *P. vulgaris* group isolates.

Aztreonam/*Pseudomonas aeruginosa*. A total of 34 *P. aeruginosa* were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 70.6%, which is not acceptable. There were 10 minor errors, no major errors, and no very major errors. Due to the unacceptable CA, this antimicrobial/organism group combination is not indicated for use with the PhAST BC GN Panel, and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
  - *Aztreonam: P. aeruginosa*

Cefepime/Enterobacterales. A total of 525 Enterobacterales (46 *C. freundii* complex, 36 *C. koseri*, 173 *E. coli*, 32 *K. aerogenes*, 27 *K. oxytoca*, 80 *K. pneumoniae* group, 41 *P. mirabilis*, 42 *S. marcescens*, 38 *E. cloacae* complex, and 10 *P. vulgaris* group) were evaluated. The susceptible-dose dependent (SDD) category is treated as an Intermediate (I) category for performance analysis. The combined results from clinical and challenge testing demonstrated a CA of 94.1%. There were 27 minor errors, 4 major errors (4/414 = 1.0%), and no very major errors. Overall performance is acceptable.

When evaluating results by individual species, *E. cloacae* complex demonstrated a CA of 81.6%, which is not acceptable. There were 6 minor errors, 1 major error (1/22 = 4.5%) and no very major errors. *P. vulgaris* group demonstrated a CA of 80.0%, which is not acceptable. Due to the unacceptable CA and major error rate of *E. cloacae* and unacceptable CA of *P. vulgaris* group, these antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
  - *Cefepime: E. cloacae complex, P. vulgaris group*

A limitation is included in the device labeling to address the lack of testing with resistant *C. koseri* isolates.

Cefepime/*Pseudomonas aeruginosa*. A total of 54 *P. aeruginosa* isolates were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 96.3%. There were 2 minor errors, no major errors, and no very major errors. Overall performance is acceptable.

Ceftazidime/Enterobacterales. A total of 553 Enterobacterales isolates (59 *E. cloacae* complex, 70 *K. aerogenes*, 28 *K. oxytoca*, 80 *K. pneumoniae*, 40 *P. mirabilis*, 42 *S. marcescens*, 27 *C. freundii* complex, 19 *C. koseri*, 176 *E. coli*, and 12 *P. vulgaris* group) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 92.6%, which is acceptable. There were 27 minor errors, 13 major errors (13/378 = 3.4%), and 1 very major error (1/164 = 0.6%).

When evaluating results by individual species, *C. freundii* complex had 1 major error (1/11 = 9.1%), which is not acceptable. *C. koseri* demonstrated a CA of 68.4%, which is not acceptable. *E. coli* demonstrated a CA of 89.8%, which is not acceptable. There were 9 minor errors, 9 major errors (9/136 = 6.6%) and no very major errors. *P. vulgaris* group demonstrated a CA of 75.0%, which is not acceptable. There were 2 minor errors, 1 major error (1/12 = 8.3%) and no very major errors. Due to the unacceptable CA and the major error rate of *E. coli* and *P. vulgaris* group, the unacceptable CA of *C. koseri*, and unacceptable major error rate of *C. freundii* complex, these antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combination(s):*
  - *Ceftazidime: C. freundii complex, C. koseri, E. coli, P. vulgaris group*

A limitation is included in the device labeling to address the lack of testing with resistant *P. mirabilis* isolates.

Ceftazidime/*Pseudomonas aeruginosa.* A total of 55 *P. aeruginosa* isolates were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 98.2%. There was 1 minor error, no major errors, and no very major errors. Overall performance is acceptable.

Ceftazidime/*Acinetobacter* spp. A total of 44 *A. baumannii* complex samples were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 100.0%. There were no minor errors, no major errors, and no very major errors. Overall performance is acceptable.

Ceftazidime/avibactam/Enterobacterales. A total of 516 Enterobacterales isolates (24 *C. freundii* complex, 19 *C. koseri*, 43 *E. cloacae* complex, 180 *E. coli*, 47 *K. aerogenes*, 28 *K. oxytoca*, 80 *K. pneumoniae*, 42 *P. mirabilis*, 11 *P. vulgaris* group and 42 *S. marcescens*) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 99.8%. There was 1 major error (1/484 = 0.2%) and no very major errors. Overall performance is acceptable.

A limitation is included in the device labeling to address the lack of testing with resistant *C. koseri*, *K. aerogenes*, *P. mirabilis*, *C. freundii* complex and *P. vulgaris* group isolates.

Ceftazidime/avibactam/*Pseudomonas aeruginosa.* A total of 55 *P. aeruginosa* isolates were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 100%. There were no major errors and no very major errors. Overall performance is acceptable.

Ceftriaxone/Enterobacterales. A total of 502 Enterobacterales isolates (26 *C. freundii* complex, 28 *C. koseri*, 43 *E. cloacae* complex, 176 *E. coli*, 32 *K. aerogenes*, 26 *K. oxytoca*, 81 *K. pneumoniae*, 40 *P. mirabilis*, 9 *P. vulgaris* group, and 41 *S. marcescens*) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 99.4%. There were no minor errors, 3 major errors (3/338 = 0.9%), and no very major errors. Overall performance is acceptable.

When evaluating results by individual species, performance *P. vulgaris* group demonstrated a CA of 88.9%, which is not acceptable. There were no minor errors, 1 major error (1/9 = 11.1%), and no very major errors. This major error is considered a random error due to the limited number of susceptible isolates tested. *S. marcescens* had 2 major errors (2/35 = 5.7%), which is not acceptable. Due to the unacceptable CA of *P. vulgaris* group and the unacceptable major error rate of *S. marcescens*, these antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
    - *Ceftriaxone: P. vulgaris group, S. marcescens*

Ciprofloxacin/Enterobacterales. A total of 547 Enterobacterales isolates (26 *C. freundii* complex, 40 *E. cloacae* complex, 217 *E. coli*, 46 *K. aerogenes*, 27 *K. oxytoca*, 78 *K. pneumoniae*, 44 *P. mirabilis*, 39 *S. marcescens*, 19 *C. koseri*, and 11 *P. vulgaris* group) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 95.2%. There were 20 minor errors, 6 major errors (6/357 = 1.7%), and no very major errors. Overall performance is acceptable.

When evaluating results by individual species, *C. koseri* demonstrated a CA of 57.9%, which is not acceptable. There were 4 minor errors, 4 major errors (4/19 = 21.1%) and no very major errors. *P. vulgaris* group demonstrated a CA of 54.5%, which is not acceptable. There were 4 minor errors, 1 major error (1/11 = 9.1%) and no very major errors. Due to the unacceptable CA and major error rate of *C. koseri* and *P. vulgaris* group, these antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
    - *Ciprofloxacin: C. koseri, P. vulgaris group*

Ciprofloxacin/*Pseudomonas aeruginosa.* A total of 51 *P. aeruginosa* isolates were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 100%. There were no major errors and no very major errors. Overall performance is acceptable.

Ertapenem/Enterobacterales. A total of 488 Enterobacterales isolates (192 *E. coli,* 41 *K. aerogenes,* 27 *K. oxytoca,* 81 *K. pneumoniae,* 42 *P. mirabilis*, 21 *C. freundii* complex, 16 *C. koseri*, 40 *E. cloacae* complex, 6 *S. marcescens*, and 22 *P. vulgaris* group) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 92.6%. There were 13 minor errors, 23 major errors (23/426 = 5.4%), and no very major errors.

When evaluating results by individual species, *C. freundii* complex demonstrated a CA of 71.4%, which is not acceptable. There were 3 minor errors, 3 major errors (3/13 = 23.1%) and no very major errors. *C. koseri* demonstrated a CA of 75.0%, which is not acceptable. There were 2 minor errors, 2 major errors (2/15 = 13.3%) and no very major errors. *E. cloacae* complex demonstrated a CA of 75.0%, which is not acceptable. There were 4 minor errors, 6 major errors (6/23 = 26.1%) and no very major errors. *S. marcescens* demonstrated a CA of 86.4%, which is not acceptable. There were no minor errors, 3 major errors (3/21 = 14.3%) and no very major errors. A limitation is included in the device labeling to address the unacceptable CA and major error rate of *C. freundii* complex, *C. koseri*, *E. cloacae* complex, and *S. marcescens*. In addition, *P. vulgaris* group met all acceptance criteria in the clinical study. However, this antimicrobial/organism combination was removed from the Indications for Use due to the concern for potential limited clinical utility as a high rate of Technical Error (TE) and No Call: Inconclusive (NC) results (53.3%) was observed during the clinical study. These antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
    - *Ertapenem: C. freundii complex, C. koseri, E. cloacae complex, S. marcescens, P. vulgaris group*

A limitation is included in the device labeling to address the lack of testing with resistant *P. mirabilis* isolates.

Gentamicin/Enterobacterales. A total of 570 Enterobacterales isolates (27 *C. freundii* complex, 18 *C. koseri,* 42 *E. cloacae* complex*,* 249 *E. coli,* 33 *K. aerogenes,* 28 *K. oxytoca,* 81 *K. pneumoniae,* 43 *P. mirabilis*, 11 *P. vulgaris* group, and 38 *S. marcescens*) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 97.4%. There were 13 minor errors, no major errors, and 2 very major errors (2/150 = 1.3%). Overall performance is acceptable.

A limitation is included in the device labeling to address the lack of testing with resistant *C. koseri*, *P. vulgaris* group and *S. marcescens* isolates.

Levofloxacin/Enterobacterales. A total of 493 Enterobacterales isolates (27 *C. freundii* complex, 19 *C. koseri,* 43 *E. cloacae* complex*,* 168 *E. coli,* 31 *K. aerogenes,* 27 *K. oxytoca,* 78 *K. pneumoniae,* 44 *P. mirabilis*, 14 *P. vulgaris* group, and 42 *S. marcescens*) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 97.0%. There were 15 minor errors, no major errors, and no very major errors. Overall performance is acceptable.

A limitation is included in the device labeling to address the lack of testing with resistant *C. koseri* and *P. vulgaris* group isolates.

Levofloxacin/*Pseudomonas aeruginosa.* A total of 51 *P. aeruginosa* isolates were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 98.0%. There was 1 minor error, no major errors, and no very major errors. Overall performance is acceptable.

Meropenem/Enterobacterales. A total of 534 Enterobacterales isolates (39 *C. freundii* complex, 36 *C. koseri,* 173 *E. coli,* 31 *K. aerogenes,* 43 *K. oxytoca,* 82 *K. pneumoniae,* 43 *P. mirabilis*, 36 *E. cloacae* complex, 12 *P. vulgaris* group, and 39 *S. marcescens*) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 95.5%. There were 4 minor errors, 14 major errors (14/481 = 2.9%), and 5 very major errors (5/52 = 9.6%).

When evaluating results by individual species, *E. cloacae* complex demonstrated a CA of 88.9%, which is not acceptable. There was 1 minor error, 2 major errors (2/24 = 8.3%) and one very major error (1/11 = 9.1%). *P. vulgaris* group demonstrated a CA of 66.7%, which is not acceptable. There were 2 minor errors, 2 major errors (2/12 = 16.7%) and no very major errors. *S. marcescens* demonstrated a CA of 82.1%, which is not acceptable. There were no minor errors, 3 major errors (3/35 = 8.6%) and 4 very major errors (4/4 = 100%). Due to the unacceptable CA, major error rate and very major error rate of *E. cloacae* complex, the unacceptable CA and major error rate of *P. vulgaris* group, and the unacceptable CA and

very major error rate of *S. marcescens*, these antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel, and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
  - *Meropenem: E. cloacae complex, P. vulgaris group, S. marcescens*

A limitation is included in the device labeling to address the lack of testing with resistant *C. koseri* and *P. mirabilis* isolates.

Meropenem/*Pseudomonas aeruginosa.* A total of 52 *P. aeruginosa* isolates were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 100%. There were no minor errors, no major errors, and no very major errors. Overall performance is acceptable.

Meropenem/*Acinetobacter* spp. A total of 42 *A. baumannii* complex samples were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 97.6%. There was 1 minor error, no major errors, and no very major errors. Overall performance is acceptable.

Piperacillin/tazobactam/Enterobacterales. A total of 497 Enterobacterales isolates (51 *E. cloacae* complex, 175 *E. coli,* 32 *K. aerogenes,* 28 *K. oxytoca*, 92 *K. pneumoniae*, 23 *C. freundii* complex, 15 *C. koseri*, 35 *P. mirabilis*, 9 *P. vulgaris* group, and 37 *S. marcescens*) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 90.7%. There were 44 minor errors, 2 major errors (2/370 = 0.5%), and no very major errors. Overall performance is acceptable.

When evaluating results by individual species, *C. koseri* demonstrated a CA of 53.3%, which is not acceptable. There were 6 minor errors, 1 major error (1/13 = 7.7%) and no very major errors. *C. freundii* complex demonstrated a CA of 87.0%, which is not acceptable. *P. mirabilis* demonstrated a CA of 88.6%, which is not acceptable. *P. vulgaris* group demonstrated a CA of 22.2% which is not acceptable. *S. marcescens* demonstrated a CA of 83.8%, which is not acceptable. Due to the unacceptable CA and the major error rate of *C. koseri*, and the unacceptable CA of *C. freundii* complex, *P. mirabilis*, *P. vulgaris* group, and *S. marcescens*, these antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel, and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
  - *Piperacillin/tazobactam: C. freundii complex, C. koseri, P. mirabilis, P. vulgaris, S. marcescens*

Piperacillin/tazobactam/*Pseudomonas aeruginosa.* A total of 52 *P. aeruginosa* isolates were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 92.3%. There were 4 minor errors, no major errors, and no very major errors. Overall performance is acceptable.

Piperacillin/tazobactam/*Acinetobacter* spp. A total of 44 *A. baumannii* complex samples were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 100%. There were no minor errors, no major errors, and no very major errors. Overall performance is acceptable.

Tobramycin/Enterobacterales. A total of 521 Enterobacterales isolates (26 *C. freundii* complex, 34 *C. koseri,* 40 *E. cloacae* complex*,* 201 *E. coli,* 32 *K. aerogenes,* 28 *K. oxytoca*, 9 *P. vulgaris* group, 78 *K. pneumoniae*, 42 *P. mirabilis*, and 31 *S. marcescens*). The combined results from clinical and challenge testing demonstrated a CA of 95.8%. There were 10 minor errors, 9 major errors (9/397 = 2.3%), and 3 very major errors (3/114 = 2.6%).

When evaluating results by individual species, *K. pneumoniae* had 2 very major errors (2/13 = 15.4%), which is not acceptable. *P. mirabilis* demonstrated a CA of 88.1%, which is not acceptable. There were 3 minor errors, 2 major errors (2/31 = 6.5%) and no very major errors. *S. marcescens* demonstrated a CA of 83.9%, which is not acceptable. There were 2 minor errors, 3 major errors (3/25 = 12.0%) and no very major errors. Due to the unacceptable CA and the very major error rate of *K. pneumoniae*, and the unacceptable CA and the major error rate of *P. mirabilis* and *S. marcescens*, these antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel, and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
    - *Tobramycin: K. pneumoniae, P. mirabilis, S. marcescens*

A limitation is included in the device labeling to address the lack of testing with resistant *C. koseri* and *P. vulgaris* group isolates.

Tobramycin/*Pseudomonas aeruginosa.* A total of 53 *P. aeruginosa* isolates were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 98.1%. There were 1 minor error, no major errors, and no very major errors. Overall performance is acceptable.

Trimethoprim/sulfamethoxazole/Enterobacterales. A total of 532 Enterobacterales isolates (16 *C. koseri*, 230 *E. coli,* 33 *K. aerogenes,* 87 *K. pneumoniae*, 38 *S. marcescens*, 21 *C. freundii* complex, 47 *E. cloacae* complex, 26 *K. oxytoca*, 38 *P. mirabilis*, and 7 *P. vulgaris* group) were evaluated. The combined results from clinical and challenge testing demonstrated a CA of 96.6%, which is acceptable. There were 18 major errors (18/386 = 4.7%) and no very major errors.

When evaluating results by individual species, *C. freundii* complex had one major error (1/10 = 10%), which is not acceptable. *E. cloacae* complex had two major errors (2/18 =11.1%), which is not acceptable. *K. oxytoca* had two major errors (2/16 = 12.5%), which is not acceptable. *P. mirabilis* demonstrated a CA of 86.8%, which is not acceptable. There were five major errors (5/20 = 25.0%) and no very major errors. A limitation is included in the device labeling to address the unacceptable major error rate of *C. freundii* complex, *E.*

cloacae complex, *K. oxytoca*, and *P. mirabilis*. In addition, performance for *P. vulgaris* group met all acceptance criteria in the clinical study. However, this antimicrobial/organism combination was removed from the Indications for Use due to the concern for potential for limited clinical utility as a high rate of TE and NC results (50.0%) was observed during the clinical study. These antimicrobial/organism combinations are not indicated for use with the PhAST BC GN Panel, and the following limitation is included in the device labeling:

- *Perform an alternative method of testing prior to reporting results for the following antibiotic/organism combinations:*
  - *Trimethoprim/sulfamethoxazole: C. freundii complex, E. cloacae complex, K. oxytoca, P. mirabilis, P. vulgaris group*

A limitation is included in the device labeling to address the lack of testing with resistant *C. koseri* isolates.

Table 8: PhAST Blood Culture Gram-Negative Panel Performance

	Total	No. CA	CA%	No. S	No. I	No. R	min	maj	vmj
Amikacin: Enterobacterales [Breakpoints: S: ≤4, I: 8, R: ≥16]									
Clinical	310	289	93.2	293	14	3	16	5	0
Challenge	198	191	96.5	164	3	31	5	1	1
Total	508	480	94.5	457	17	34	21	6	1
[a]Ampicillin/sulbactam: Enterobacterales [Breakpoints: S: ≤8/4, I: 16/8, R: ≥32/16]									
Clinical	244	132	54.1	206	27	11	51	61	0
Challenge	88	63	71.6	45	5	38	18	7	0
Total	332	195	58.7	251	32	49	69	68	0
Ampicillin/sulbactam: *Acinetobacter* spp. [Breakpoints: S: ≤8/4, I: 16/8, R: ≥32/16]									
Clinical	1	1	100	1	0	0	0	0	0
Challenge	61	56	91.8	34	3	24	4	1	0
Total	62	57	91.9	35	3	24	4	1	0
Aztreonam: Enterobacterales [Breakpoints: S: ≤4, I: 8, R: ≥16]									
Clinical	332	320	96.4	265	7	60	10	2	0
Challenge	162	157	96.9	92	4	66	4	1	0
Total	494	477	96.6	357	11	126	14	3	0
[a]Aztreonam: *P. aeruginosa* [Breakpoints: S: ≤8, I: 16, R: ≥32]									
Clinical	3	2	66.7	3	0	0	1	0	0
Challenge	31	22	71.0	11	9	11	9	0	0
Total	34	24	70.6	14	9	11	10	0	0
Cefepime: Enterobacterales [Breakpoints: S: ≤2, SDD: 4-8, R: ≥16]									
Clinical	324	298	92.0	271	21	32	22	4	0
Challenge	201	196	97.5	143	5	53	5	0	0
Total	525	494	94.1	414	26	85	27	4	0
Cefepime: *P. aeruginosa* [Breakpoints: S: ≤8, I: 16, R: ≥32]									
Clinical	13	13	100	13	0	0	0	0	0
Challenge	41	39	95.1	12	1	28	2	0	0
Total	54	52	96.3	25	1	28	2	0	0
Ceftazidime: Enterobacterales [Breakpoints: S: ≤4, I: 8, R: ≥16]									
Clinical	337	298	88.4	269	11	57	25	13	1
Challenge	216	214	99.1	109	0	107	2	0	0
Total	553	512	92.6	378	11	164	27	13	1
Ceftazidime: *P. aeruginosa* [Breakpoints: S: ≤8, I: 16, R: ≥32]									
Clinical	14	14	100	14	0	0	0	0	0
Challenge	41	40	97.6	12	1	28	1	0	0
Total	55	54	98.2	26	1	28	1	0	0

Ceftazidime: *Acinetobacter* spp. [Breakpoints: S: ≤8, I: 16, R: ≥32]									
Clinical	1	1	100	0	0	1	0	0	0
Challenge	43	43	100	15	0	28	0	0	0
Total	44	44	100	15	0	29	0	0	0
Ceftazidime/avibactam: Enterobacterales [Breakpoints: S: ≤8/4, R: ≥16/4]									
Clinical	327	326	99.7	325	n/a	2	n/a	1	0
Challenge	178	178	100	148	n/a	30	n/a	0	0
Total	516	515	99.8	484	n/a	32	n/a	1	0
Ceftazidime/avibactam: *P. aeruginosa* [Breakpoints: S: ≤8/4, R: ≥16/4]									
Clinical	14	14	100	14	n/a	0	n/a	0	0
Challenge	41	41	100	12	n/a	29	n/a	0	0
Total	55	55	100	26	n/a	29	n/a	0	0
Ceftriaxone: Enterobacterales [Breakpoints: S: ≤1, I: 2, R: ≥4]									
Clinical	328	325	99.1	243	0	85	0	3	0
Challenge	174	174	100	95	0	79	0	0	0
Total	502	499	99.4	338	0	164	0	3	0
Ciprofloxacin: Enterobacterales [Breakpoints: S: ≤0.25, I: 0.5, R: ≥1]									
Clinical	313	289	92.3	237	3	73	19	5	0
Challenge	234	232	99.1	120	1	113	1	1	0
Total	547	521	95.2	357	4	186	20	6	0
Ciprofloxacin: *P. aeruginosa* [Breakpoints: S: ≤0.5, I: 1, R: ≥2]									
Clinical	10	10	100	10	0	0	0	0	0
Challenge	41	41	100	12	0	29	0	0	0
Total	51	51	100	22	0	29	0	0	0
Ertapenem: Enterobacterales [Breakpoints: S: ≤0.5, I: 1, R: ≥2]									
Clinical	295	266	90.2	284	2	9	10	19	0
Challenge	193	186	96.4	142	3	48	3	4	0
Total	488	452	92.6	426	5	57	13	23	0
Gentamicin: Enterobacterales [Breakpoints: S: ≤2, I: 4, R: ≥8]									
Clinical	336	323	96.1	297	0	39	11	0	2
Challenge	234	232	99.1	121	2	111	2	0	0
Total	570	555	97.4	418	2	150	13	0	2
Levofloxacin: Enterobacterales [Breakpoints: S: ≤0.5, I: 1, R: ≥2]									
Clinical	332	319	96.1	262	7	63	13	0	0
Challenge	161	159	98.8	99	0	62	2	0	0
Total	493	478	97.0	361	7	125	15	0	0
Levofloxacin: *P. aeruginosa* [Breakpoints: S: ≤1, I: 2, R: ≥4]									
Clinical	12	11	91.7	12	0	0	1	0	0
Challenge	39	39	100	10	0	29	0	0	0
Total	51	50	98.0	22	0	29	1	0	0
Meropenem: Enterobacterales [Breakpoints: S: ≤1, I: 2, R: ≥4]									
Clinical	321	304	94.7	316	0	5	3	12	2
Challenge	213	206	96.7	165	1	47	1	2	3
Total	534	510	95.5	481	1	52	4	14	5
Meropenem: *P. aeruginosa* [Breakpoints: S: ≤2, I: 4, R: ≥8]									
Clinical	12	12	100	12	0	0	0	0	0
Challenge	40	40	100	12	0	28	0	0	0
Total	52	52	100	24	0	28	0	0	0
Meropenem: *Acinetobacter* spp. [Breakpoints: S: ≤2, I: 4, R: ≥8]									
Clinical	1	0	0.0	0	1	0	1	0	0
Challenge	41	41	100	15	0	26	0	0	0
Total	42	41	97.6	15	1	26	1	0	0
Piperacillin/tazobactam: Enterobacterales [Breakpoints: S: ≤8/4, I: 16/4, R: ≥32/4]									
Clinical	301	260	86.4	266	11	24	39	2	0
Challenge	196	191	97.4	104	1	91	5	0	0
Total	497	451	90.7	370	12	115	44	2	0

Piperacillin/tazobactam: *P. aeruginosa* [Breakpoints: S: ≤16/4, I: 32/4, R: ≥64/4]									
Clinical	13	12	92.3	13	0	0	1	0	0
Challenge	39	36	92.3	12	2	25	3	0	0
Total	52	48	92.3	25	2	25	4	0	0
Piperacillin/tazobactam: *Acinetobacter* spp. [Breakpoints: S: ≤16/4, I: 32/4-64/4, R: ≥128/4]									
Clinical	1	1	100	0	0	1	0	0	0
Challenge	43	43	100	15	0	28	0	0	0
Total	44	44	100	15	0	29	0	0	0
Tobramycin: Enterobacterales [Breakpoints: S: ≤2, I: 4, R: ≥8]									
Clinical	310	294	94.8	269	5	36	5	8	3
Challenge	211	205	97.2	128	5	78	5	1	0
Total	521	499	95.8	397	10	114	10	9	3
Tobramycin: *P. aeruginosa* [Breakpoints: S: ≤1, I: 2, R: ≥4]									
Clinical	13	12	92.3	13	0	0	1	0	0
Challenge	40	40	100	12	0	28	0	0	0
Total	53	52	98.1	25	0	28	1	0	0
Trimethoprim/sulfamethoxazole: Enterobacterales [Breakpoints: S: ≤2/38, R: ≥4/76]									
Clinical	301	290	96.3	225	n/a	76	n/a	11	0
Challenge	231	224	97.0	161	n/a	70	n/a	7	0
Total	532	514	96.6	386	n/a	146	n/a	18	0

[a] Due to unacceptable performance, do not report PhAST BC GN Panel results for these antimicrobial/organism combinations.
*SDD- Susceptible-Dose Dependent

CA - Category Agreement

R - Resistant isolates
I - Intermediate isolates
S - Susceptible isolates

min - Minor Discrepancies
maj - Major Discrepancies
vmj - Very Major Discrepancies

Category Agreement (CA) occurs when the interpretation of the reference method and PhAST BC GN Panel result are in exact agreement.

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable

2. Clinical Specificity:

Not applicable

3. Clinical Cut-Off

Not applicable

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

Not applicable

D Expected Values/Reference Range:

The PhAST BC GN Panel with the PhAST instrument reports a categorical interpretation (Susceptible, Intermediate/Susceptible-Dose Dependent, Resistant) in accordance with the FDA Susceptibility Test Interpretive Criteria in the table below.

Table 9. FDA-Approved or Recognized Interpretive Criteria[a]

Antimicrobial	Organism	Minimum Inhibitory Concentration (µg/mL)		
		Susceptible	Intermediate	Resistant
Amikacin	Enterobacterales	≤4	8	≥16
Ampicillin/sulbactam	*Acinetobacter* spp.	≤8/4	16/8	≥32/16
	Enterobacterales	≤8/4	16/8	≥32/16
Aztreonam	Enterobacterales	≤4	8	≥16
	Pseudomonas aeruginosa	≤8	16	≥32
Cefepime	Enterobacterales	≤2	4-8[b]	≥16
	Pseudomonas aeruginosa	≤8	16	≥32
Ceftazidime	*Acinetobacter* spp.	≤8	16	≥32
	Enterobacterales	≤4	8	≥16
	Pseudomonas aeruginosa	≤8	16	≥32
Ceftazidime/avibactam	Enterobacterales	≤8/4	-	≥16/4
	Pseudomonas aeruginosa	≤8/4	-	≥16/4
Ceftriaxone	Enterobacterales	≤1	2	≥4
Ciprofloxacin	Enterobacterales	≤0.25	0.5	≥1
	Pseudomonas aeruginosa	≤0.5	1	≥2
Ertapenem	Enterobacterales	≤0.5	1	≥2
Gentamicin	Enterobacterales	≤2	4	≥8
Levofloxacin	Enterobacterales	≤0.5	1	≥2
	Pseudomonas aeruginosa	≤1	2	≥4
Meropenem	*Acinetobacter* spp.	≤2	4	≥8
	Enterobacterales	≤1	2	≥4
	Pseudomonas aeruginosa	≤2	4	≥8
Piperacillin/tazobactam	*Acinetobacter* spp.	≤16/4	32/4-64/4	≥128/4
	Enterobacterales	≤8/4	16/4	≥32/4
	Pseudomonas aeruginosa	≤16/4	32/4	≥64/4
Tobramycin	Enterobacterales	≤2	4	≥8
	Pseudomonas aeruginosa	≤1	2	≥4
Trimethoprim/ sulfamethoxazole	Enterobacterales	≤2/38	-	≥4/76

[a]According to FDA STIC Webpage, https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria

[b]SDD, Susceptible-Dose Dependent category is reported for Cefepime/Enterobacterales

E Other Supportive Instrument Performance Characteristics Data:

EMC: Electrical safety and electromagnetic compatibility (EMC) testing were performed, and the system was found to be acceptable.
Software and cybersecurity: Software and cybersecurity documentation were reviewed and found to be acceptable.

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.