EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Accelerate PhenoTest BC Kit

DECISION SUMMARY

A. DEN Number:

DEN160032

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation for the Accelerate PhenoTest BC Kit.

C. Measurand:

The following Gram-positive and Gram-negative bacteria and yeasts are identified using the Accelerate PhenoTest BC kit *Staphylococcus aureus, Staphylococcus lugdunensis*, Coagulase-negative *Staphylococcus* species (i.e., *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus lugdunensis*, *Staphylococcus warnerii*, not differentiated), *Enterococcus faecalis, Enterococcus faecium*, *Streptococcus* spp. (i.e., *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus gallolyticus, Streptococcus agalactiae, Streptococcus pneumoniae*, not differentiated), *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella* spp. (i.e., *Klebsiella pneumoniae, Klebsiella oxytoca*, not differentiated*), Escherichia coli, Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis, Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii, Citrobacter koseri*, not differentiated*), Serratia marcescens, Candida albicans* and *Candida glabrata.*

The Accelerate PhenoTest BC kit tests the following antimicrobial agents as appropriate for the identified organism (*see* section H below): amikacin, ampicillin, ampicillin/sulbactam, aztreonam, ceftazidime, ceftaroline, cefepime, ceftriaxone, ciprofloxacin, daptomycin, erythromycin, ertapenem, gentamicin, linezolid, meropenem, piperacillin/tazobactam, tobramycin and vancomycin.

The following resistance phenotypes are reported based on qualitative tests as appropriate for the identified organism (*see* section H below): Methicillin-resistance and macrolide-lincosamide-streptogramin B resistance (MLSb).

D. Type of Test:
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 PhenoTest BC assay is performed directly on positive blood culture samples identified as positive by a continuous monitoring blood culture system.

E. Applicant:
Accelerate Diagnostics

F. Proprietary and Established Names:
Accelerate PhenoTest BC Kit

Accelerate Pheno system

G. Regulatory Information:

1. Regulation section:
866.1650

2. Classification:
Class II

3. Product code:
PRH, NSU, PEO, PAM, PEN, LON

4. Panel:
83 (Microbiology)

H. Indications for Use:

1. Indications for Use:

The Accelerate PhenoTest BC kit is a multiplexed *in vitro* diagnostic test utilizing both qualitative nucleic acid fluorescence *in situ* hybridization (FISH) identification and quantitative, antimicrobial susceptibility testing (AST) methods and is intended for use with the Accelerate Pheno system. The Accelerate PhenoTest BC kit is capable of simultaneous detection and identification of multiple microbial targets followed by susceptibility testing of the appropriate detected bacterial organisms. The Accelerate PhenoTest BC kit is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system. Results are intended to be interpreted in conjunction with Gram stain results.

The Accelerate PhenoTest BC kit identifies the following Gram-positive and Gram-negative bacteria and yeasts utilizing FISH probes targeting organism-specific ribosomal RNA sequences: *Staphylococcus aureus*, *Staphylococcus lugdunensis*, Coagulase-negative S*taphylococcus* species (i.e., *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis, Staphylococcus capitis, Staphylococcus lugdunensis, Staphylococcus warneri,* not differentiated), *Enterococcus faecalis, Enterococcus faecium, Streptococcus* spp. (i.e., *Streptococcus mitis, Streptococcus oralis, Streptococcus gallolyticus, Streptococcus agalactiae, Streptococcus pneumoniae*, not differentiated), *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Klebsiella* spp. (i.e., *Klebsiella pneumoniae, Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae, Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis, Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii, Citrobacter koseri*, not differentiated), *Serratia marcescens, Candida albicans* and *Candida glabrata.*

The Accelerate PhenoTest BC kit tests the following antimicrobial agents with the specific target organisms identified below:

- Amikacin: *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella* spp. (i.e., *Klebsiella pneumoniae, Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, Enterobacter *aerogenes*, not differentiated), Proteus *spp*. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*

- Ampicillin: *Enterococcus faecalis* and *Enterococcus faecium*
- Ampicillin/Sulbactam: *Escherichia coli*, *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), and *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated)
- Aztreonam: *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*
- Ceftazidime: *Pseudomonas aeruginosa*, *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and Serratia *marcescens*
- Ceftaroline : *Staphylococcus aureus*
- Cefepime: *Pseudomonas aeruginosa*, *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*
- Ceftriaxone: *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*
- Ciprofloxacin: *Pseudomonas aeruginosa*, *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*
- Daptomycin: *Staphylococcus aureus*, Coagulase-negative *Staphylococcus* species (i.e., *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus lugdunensis*, *Staphylococcus warneri*, not differentiated), *Enterococcus faecalis* and *Enterococcus faecium*
- Erythromycin: *Staphylococcus aureus*
- Ertapenem: *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*
- Gentamicin: *Pseudomonas aeruginosa*, *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*
- Linezolid: *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*
- Meropenem: *Pseudomonas aeruginosa*, *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not

differentiated) and *Serratia marcescens*

- Piperacillin/Tazobactam: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*
- Tobramycin: *Pseudomonas aeruginosa*, *Klebsiella* spp. (i.e., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, not differentiated), *Escherichia coli*, *Enterobacter* spp. (i.e., *Enterobacter cloacae*, *Enterobacter aerogenes*, not differentiated), *Proteus* spp. (i.e., *Proteus mirabilis*, *Proteus vulgaris*, not differentiated), *Citrobacter* spp. (i.e., *Citrobacter freundii*, *Citrobacter koseri*, not differentiated) and *Serratia marcescens*
- Vancomycin: *Staphylococcus aureus*, *Staphylococcus lugdunensis*, Coagulase-negative *Staphylococcus* species (i.e., *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus lugdunensis*, *Staphylococcus warneri*, not differentiated), *Enterococcus faecalis* and *Enterococcus faecium*

The following resistance phenotypes are reported based on qualitative tests: Methicillin-resistance (*S. aureus S. lugdunensis*, coagulase negative staphylococci) and macrolide-lincosamide-streptogramin B resistance (MLSb) (*S. lugdunensis* and coagulase negative staphylococci).

The Accelerate PhenoTest BC kit is indicated as an aid in the diagnosis of bacteremia and fungemia. It is also indicated for susceptibility testing of specific pathogenic bacteria as identified above commonly associated with or causing bacteremia. 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 identification and susceptibility testing of: organisms not identified by the Accelerate PhenoTest BC kit, organisms present in polymicrobial samples, organisms for which species identification is critical for patient care (e.g., speciation of *Streptococcus* spp.), samples for which an "indeterminate" result for any probe was obtained, for testing antimicrobial agents not included on the Accelerate panel and for epidemiologic testing.

2. Special conditions for use statement(s):
   For prescription use only

   Limitations:

   General Limitations
   - This product can only be used with the Accelerate Pheno system.
   - The Accelerate PhenoTest BC kit assay has not been evaluated for specimens other than blood (e.g., sterile body fluids inoculated into blood culture bottles)
   - The performance of this test has only been evaluated using the following blood culture bottles:
     - BD BACTEC Standard/10 Aerobic/F Medium,
     - BD BACTEC Standard/10 Anaerobic/F Medium,
     - BD BACTEC Lytic/10 Anaerobic/F Medium
     - BD BACTEC PEDS PLUS/F Medium
     - BD BACTEC Plus Aerobic/F Medium
     - BD BACTEC Plus Anaerobic/F Medium

    - BioMérieux BacT/ALERT SA Standard Aerobic
    - BioMérieux BacT/ALERT SN Standard Anaerobic
    - BioMérieux BacT/ALERT FA Plus Aerobic
    - BioMérieux BacT/ALERT FN Plus Anaerobic
    - BioMérieux BacT/ALERT PF Plus
    - Versa TREK REDOX 1 (Aerobic) Medium and
    - Versa TREK REDOX 2 (Anaerobic) Medium
- This product should not be used with blood culture bottles containing charcoal.
- Positive blood culture samples must be run using the Accelerate PhenoTest BC kit on the Accelerate Pheno system within 8 hours of sample positivity.
- Positive blood culture sample must be loaded on the Accelerate Pheno system and the run must be initiated within 15 minutes of pipetting sample into sample vial and within 1 hour of removing the assay kit from refrigerated storage.
- Failure to observe proper procedures for sample collection, preparation, storage, handling and/or transportation may cause incorrect results.

Identification (ID) Limitations

- Due to the possibility of cross reactivity, all Accelerate PhenoTest BC kit results should be interpreted in conjunction with Gram stain.
- The ability of probes to detect all strains of a target species was not predicted by *in-silico* analysis.
- Additional subculture is required for the identification of *S. pneumoniae* in cases of a positive *Streptococcus* spp. call.
- Subculture of positive blood culture is required in the following situations:
    - For the identification and susceptibility testing of off-panel organisms not identified by the Accelerate PhenoTest BC kit,
    - For samples that give a polymicrobial result
    - For organisms for which species identification is critical for patient care, (e.g., speciation of streptococci)
    - For testing antimicrobial agents not included on the Accelerate panel
    - For testing certain antimicrobial agents as discussed in AST limitations below
    - For testing samples for which an “indeterminate” result for any probe was obtained
    - To obtain isolates for epidemiologic testing.
- Accelerate PhenoTest BC kit identification results that are discordant with the result of the blood culture Gram stain (for example, no organism detection when the Gram stain is positive or detection of a Gram-positive cocci when Gram-positive cocci were not observed in the Gram stain) should be confirmed by an alternate technique prior to reporting the test result. For some polymicrobic calls, false positive results may not be mitigated by Gram stain analysis (for example, detection of two Enterobacteriaceae species with Gram-negative rods observed in the Gram stain). Results of such polymicrobic calls should be verified by subculture and/or an alternative identification method.

Antimicrobial Susceptibility Testing (AST) Limitations

- Due to insufficient number of test isolates, the ability of the Accelerate PhenoTest BC kit to detect inducible MLSb resistance in coagulase-negative staphylococci is unknown when used with the following blood culture bottle types: BacT/Alert SN Standard Anaerobic, BACTEC Peds Plus/F, BACTEC Plus Anaerobic/F, BACTEC Standard Anaerobic, BACTEC Standard/10 Aerobic, VersaTrek Redox 1 Aerobic, VersaTrek

Redox 2 Anaerobic. Use an alternative method for detection of inducible MLSb resistance when using these blood culture bottle types if critical to patient care.

- Susceptibility testing of monomicrobial samples will only be performed when on-panel species eligible for susceptibility testing are detected. See intended use.
- Susceptibility testing of polymicrobial samples will only be performed on one organism out of a pair of species that meet all of the following criteria:
  - One or both organisms must be on the Accelerate PhenoTest BC kit test panel and eligible for AST testing (except *Proteus* spp.)
  - The two organisms must have distinct growth responses or morphological differences such that growing clones from each species can be differentiated by the software. These include the following pairs:

Polymicrobial Testing Pairs

Organism 1	Organism 2
• AST-eligible organism (except *Proteus* spp.)	• AST-ineligible organism
• *Escherichia coli* • *Klebsiella* spp. • *Enterobacter* spp. • *Citrobacter* spp. or • *Serratia marcescens*	• *Staphylococcus aureus* • Coagulase-negative *Staphylococcus* spp. • *Enterococcus* spp. *(E. faecalis, E. faecium)* or • *Pseudomonas aeruginosa*
• *Acinetobacter baumannii*	• *Enterococcus* spp. *(E. faecalis, E. faecium)*

  - Only one organism when diluted for AST must be within the required concentration limits for AST testing (10-130 growing clones per field of view).

    If the concentration ratio between organisms is such that only one organism could be diluted to the concentration range required for AST testing, AST testing will only be performed on the higher concentration organism. If one of the organisms in the pair is eligible for AST testing and the other is not, AST testing will only be performed on the on-panel AST-eligible organism. If both organisms are eligible for AST testing and are within the required concentration limits for AST testing, AST results will not be reported.

- If an AST result is not provided by the Accelerate PhenoTest BC kit, susceptibility testing must be performed using an alternative method.
- Subculturing of positive blood culture is necessary for the identification and susceptibility testing of organisms not identified by the Accelerate PhenoTest BC kit and for antimicrobial agents not included on the Accelerate panel.
- Potential interference by antimicrobial agents that may be present in a patient blood specimen has not been established with the Accelerate PhenoTest BC kit.
- The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combinations is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing:
  - Amikacin: *Citrobacter* spp., *Enterobacter* spp., *E. coli, Proteus* spp., *S. marcescens*

- Aztreonam: *Proteus* spp.*, S. marcescens*
    - Cefepime: *Citrobacter* spp.*, Proteus* spp.*, S. marcescens*
    - Ceftazidime: *Proteus* spp.*, S. marcescens*
    - Ceftaroline: *S. aureus*
    - Ceftriaxone: *Citrobacter* spp.*, E. cloacae, S. marcescens*
    - Ciprofloxacin: *Citrobacter* spp.*, Proteus* spp.*, S. marcescens*
    - Daptomycin: *S. aureus*
    - Ertapenem: *Citrobacter* spp.*, Proteus* spp.*, and S. marcescens*
    - Gentamycin: *Citrobacter* spp.*, Enterobacter* spp.*, Proteus* spp.*, S. marcescens*
    - Meropenem: *Citrobacter* spp.*, E. coli, Proteus* spp.*, and S. marcescens*
    - Piperacillin/Tazobactam: *Proteus* spp.*, and S. marcescens*
    - Tobramycin: *Citrobacter* spp.*, Proteus* spp.*, S. marcescens*
    - Cefoxitin for Phenotypic Resistance: *S.lugdunensis*
    - MLSb: *S. lugdunensis*
- The following antimicrobial/organism combinations may produce a resistant result that can be found susceptible by the reference method. If critical to patient care confirm these results with an alternate method:
    - Meropenem: *Enterobacter*
    - Ceftazidime: *Pseudomonas aeruginosa* (Any *P. aeruginosa* isolate that provides an MIC ≥16 µg/mL should be retested using an alternate method)
    - Cefepime: *Pseudomonas aeruginosa*
    - Ertapenem: *Enterobacter* spp.
    - Piperacillin/Tazobactam: *Acinetobacter baumannii*, *Klebsiella* spp.
- The ability of the Accelerate PhenoTest BC kit to provide accurate MICs with amikacin resistant strains of *A. baumannii* has not been established; isolates of this species that provide resistant results should be confirmed by an alternative method.
- Due to a low essential agreement for *Serratia marcescens* with ceftriaxone, results should be confirmed with an alternate method if critical to patient care.
- The current absence of data on daptomycin-resistant isolates precludes defining any categories other than "susceptible." Isolates yielding test results suggestive of a non-susceptible category should be retested and if the result is confirmed, the isolate should be retested using the reference method.
- The ability of the Accelerate PhenoTest BC kit to detect vancomycin-intermediate Staphylococcus aureus isolates (VISA) is unknown because insufficient numbers of VISA isolates were evaluated at the time of comparative testing.
- Any *S. aureus* isolate for which the vancomycin MIC is >= 8 µg/mL should be sent to a reference laboratory for reference method testing.
- Any coagulase negative Staphylococcus isolate for which the vancomycin MIC is >= 32 µg/mL should be sent to a reference laboratory for reference method testing.
- The ability of the Accelerate PhenoTest BC kit to provide accurate results for *S. aureus* with MLSb as compared to the reference method has not been established; isolates of this species should be tested by an alternative method.

3. <u>Special instrument requirements:</u>

   The Accelerate PhenoTest BC Kit is performed on the Accelerate Pheno System.

I. Device Description:

The Accelerate Pheno system is comprised of the Accelerate Pheno instrument, software, host computer, analysis computer, and the Accelerate PhenoTest BC kit. The Accelerate PhenoTest BC

Kit contains a sample vial, a 48-channel disposable test cassette and a reagent cartridge needed to test samples from a blood culture bottle that has been flagged as positive by a continuous monitoring blood culture system. All identification (ID) and antimicrobial susceptibility testing (AST) is performed in individual flowcells of the test cassette. The reagent cartridge contains gel electrofiltration (GEF) stations, fluorescence *in situ* hybridization (FISH) probes, antibiotics, and reagents for automated sample preparation, identification of bacterial and fungal target organisms (Table 1), and antimicrobial susceptibility testing and phenotypic resistance detection testing for bacterial target organisms (Tables 2 and 3). The user loads an aliquot of the positive blood culture into the sample vial, places the test cassette, reagent cartridge and sample vial into an Accelerate Pheno System module, and then presses the module button to close the module door and start the run. The remainder of the operations are automated as described below.

Automated Sample Preparation
Automated sample preparation is performed using gel electro-filtration (GEF) which is based on gel electrophoresis principles. The sample is automatically transferred to a gel containing pores smaller than bacterial or yeast cells. Application of an electric field causes lysed blood cells and/or other sample debris to pass into the gel while bacterial/yeast cells remain inside the gel well. The electric field is briefly reversed to dislodge bacterial/yeast cells from the gel wall prior to removal.

Cell Capture
Following sample preparation, recovered cells are automatically pipetted into multiple flowcell channels of the test cassette. Conductive layers of transparent indium tin oxide (ITO) coat the top and bottom inner surfaces of each flowcell channel and act as electrodes. An additional cationic poly-L-lysine layer on the bottom of each flowcell acts as a capture surface. When a voltage is applied, the negatively-charged bacterial/yeast cells migrate to the positively-charged capture surface where they are captured prior to imaging.

Fluorescence *in situ* Hybridization (FISH) for Identification
Cocktails of ATTO-532 (green) fluorescently-labeled DNA probes bind to the ribosomal RNA of target organisms following permeabilization. Each cocktail also includes ATTO-647 (red) labeled universal bacterial probe that binds to the ribosomal RNA of all clinically relevant bacteria (bacterial ID channels) or universal eukaryotic probe that binds to the ribosomal RNA of all clinically relevant yeast (yeast ID channels). The system images each flowcell using an epifluorescence microscope with camera at 532 nm, 647 nm and in dark-field. To exclude debris, only dark-field objects that are colocalized with universal probe signal are included in analysis. Colocalization of target probe signal and universal probe signal identifies a target organism.

The software also quantitates the total number of organisms present in a sample using a nucleic acid stain in a separate control flowcell. Comparing the relative numbers of each target organism to the number of objects lit up by the universal probes and universal nucleic acid stain allows for non-target organism and polymicrobial sample detection. FISH ID results are reported approximately 90 minutes after loading the sample, and the ID result determines the selection of appropriate antibiotics for subsequent antimicrobial susceptibility testing.

Table 1. Probe Sets and Species Identified by Each Probe Set

Probe Set	On-panel Species
ABA	*Acinetobacter baumannii*
CAL	*Candia albicans*
CGL	*Candida glabrata*
CIT	*Citrobacter freundii*

Probe Set	On-panel Species
	Citrobacter koseri
CNS	*Staphylococcus epidermidis* *Staphylococcus haemolyticus* *Staphylococcus hominis* *Staphylococcus capitis* *Staphylococcus lugdunensis* *Staphylococcus warneri*
ECO	*Escherichia coli*
EFM	*Enterococcus faecium*
EFS	*Enterococcus faecalis*
ENT	*Enterobacter aerogenes* *Enterobacter cloacae*
KLE	*Klebsiella oxytoca* *Klebsiella pneumoniae*
PAE	*Pseudomonas aeruginosa*
PRO	*Proteus mirabilis* *Proteus vulgaris*
SAU	*Staphylococcus aureus*
SLU	*Staphylococcus lugdunensis*
SMA	*Serratia marcescens*
STR	*Streptococcus mitis* *Streptococcus oralis* *Streptococcus gallolyticus* *Streptococcus agalactiae* *Streptococcus pneumoniae*

Morphokinetic Cellular Analysis (MCA) for Antimicrobial Susceptibility Testing (AST)
Sample remaining after the identification assay is initiated is combined with growth media and organisms contained in the sample undergo a pre-growth step during the FISH ID assay to normalize growth rates prior to AST. Following automated sample preparation and cell capture, growth media containing single concentrations of each test antibiotic are added to separate flowcell channels; antibiotics are selected based on the identification provided by the FISH identification (Tables 2 and 3). The bacteria in each flowcell are imaged every 10 minutes for up to 4.5 hours, creating a time-lapse record of bacterial growth from individual progenitor cells into clones of daughter cells.

During this period, several microscopic features are measured through morphokinetic cellular analysis, such as cell morphology and the light intensity of a growing clone over time, and used for analysis. The precise quantitative measurement of individual clone growth rate over time is an indicator of antimicrobial efficacy. Onboard software algorithms derive minimum inhibitory concentration (MIC) values from the measured features, and apply appropriate expert rules for proper interpretation and reporting of categorical interpretations - S, I or R (susceptible, intermediate, or resistant) for MIC determinations and positive or negative for phenotypic resistance markers. AST results are reported in approximately 5 hours after ID results. The reportable ranges for each antimicrobial and phenotypic resistance markers are listed in Tables 4 and 5.

Algorithm for Performance of AST

For samples determined to be monomicrobic (only one pathogen detected), susceptibility testing will only be performed when one of the on-panel species eligible for susceptibility testing are detected. The following limitation is included in the device labeling:

> *Susceptibility testing of monomicrobial samples will only be performed when on-panel species eligible for susceptibility testing are detected.*

For samples determined to be polymicrobic, susceptibility testing will only be performed on one organism out of a pair of species that meet all of the following criteria:

- One or both organisms must be on the Accelerate PhenoTest BC kit test panel and eligible for AST testing (except *Proteus* spp.)
- The two organisms must have distinct growth responses or morphological differences such that growing clones from each species can be differentiated by the software. These include the following pairs:

Organism 1	Organism 2
• AST-eligible organism (except *Proteus* spp.)	• AST-ineligible organism
• *Escherichia coli* • *Klebsiella* spp. • *Enterobacter* spp. • *Citrobacter* spp. or • *Serratia marcescens*	• *Staphylococcus aureus* • Coagulase-negative *Staphylococcus* spp. • *Enterococcus* spp. *(E. faecalis, E. faecium* or other *Enterococcus* spp.*)* or • *Pseudomonas aeruginosa*
• *Acinetobacter baumannii*	• *Enterococcus* spp. *(E. faecalis, E. faecium)*

- Only one organism when diluted for AST must be within the required concentration limits for AST testing (10-130 growing clones per field of view).

If the concentration ratio between organisms is such that only one organism could be diluted to the concentration range required for AST testing, AST testing will only be performed on the higher concentration organism. If one of the organisms in the pair is eligible for AST testing and the other is not, AST testing will only be performed on the on-panel AST-eligible organism. If both organisms are eligible for AST testing and are within the required concentration limits for AST testing, AST results will not be reported.

Because the PhenoTest BC Kit will not be capable of performing AST testing on all organisms present in a positive blood culture, subculture is required to recover the additional organism(s) and to allow performance of AST by an alternate method. In addition, subculture is required to recover organisms not detected by the PhenoTest BC Kit and for testing antimicrobial agents not included in the kit. The following limitations are included in the device labeling:

> *If an AST result is not provided by the Accelerate PhenoTest BC kit, susceptibility testing must be performed using an alternative method.*

Subculture of positive blood culture is required in the following situations:

- *For testing antimicrobial agents not included on the Accelerate Panel*
- *For testing certain antimicrobial agents as discussed in AST limitations.*

Table 2. Antimicrobial Agents Tested*, Gram-Positive Organisms

Organism	Ampicillin	Ceftaroline	Erythromycin	Daptomycin	Linezolid	Vancomycin	Cefoxitin	MLSb
S. aureus	-	X	X	X	X	X	X	-
S. lugdunensis	-	-	-	-	-	X	X	X
Coagulase negative *Staphylococcus*	-	-	-	X	-	X	X	X
Enterococcus faecalis	X	-	-	X	X	X	-	-
Enterococcus faecium	X	-	-	X	X	X	-	-

*(X) = Tested, (-) = Not tested

Table 3. Antimicrobial Agents Tested*, Gram-Negative Organisms

Organism	Ampicillin-sulbactam	Piperacillin-tazobactam	Cefepime	Ceftazidime	Ceftriaxone	Ertapenem	Meropenem	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Aztreonam
E. coli	X	X	X	X	X	X	X	X	X	X	X	X
***Klebsiella* spp.**	X	X	X	X	X	X	X	X	X	X	X	X
***Enterobacter* spp.**	-	X	X	X	X	X	X	X	X	X	X	X
***Proteus* spp.**	X	X	X	X	X	X	X	X	X	X	X	X
***Citrobacter* spp.**	-	X	X	X	X	X	X	X	X	X	X	X
S. marcescens	-	X	X	X	X	X	X	X	X	X	X	X
P. aeruginosa	-	X	X	X	-	-	X	X	X	X	X	X
A. baumannii	-	X	-	-	-	-	-	X	-	-	-	-

*(X) = Tested, (-) = Not tested

Table 4. Reportable MIC Ranges and Organism-Specific Breakpoints for Antimicrobials Included in the PhenoTest BC

Antimicrobial	Organisms Tested	Reportable Range in PhenoTest BC (µg/mL)	FDA Breakpoints		
			S	I	R
Gram-Positive					
Ampicillin	*Enterococcus spp.*	≤ 2 to ≥ 32	≤ 8	-	≥ 16
Ceftaroline	*S. aureus*	≤ 0.25 to ≥ 8	≤ 1	2	≥ 4
Daptomycin	*Staphylococcus spp.*	≤ 0.25 to ≥ 4	≤ 1	-	≥ 2
	Enterococcus spp.	≤ 1 to ≥ 16	≤ 4	-	≥ 8
Erythromycin	*S. aureus*	≤0.125 to ≥ 16	≤0.5	1 - 4	≥ 8
Linezolid	*S. aureus*	≤ 1 to ≥ 16	≤ 4	-	≥ 8
	Enterococcus spp.	≤ 0.5 to ≥ 16	≤ 2	4	≥ 8
Vancomycin	*S. aureus*	≤ 0.5 to ≥ 32	≤ 2	4 - 8	≥ 16
	S. lugdunensis and Coagulase Negative *Staphylococcus*	≤ 1 to ≥ 64	≤ 4	8-16	≥ 32
Gram-Negative					
Amikacin	*Enterobacteriaceae*	≤ 4 to ≥ 128	≤ 16	32	≥ 64
	P. aeruginosa				
	A. baumannii				
Ampicillin/Sulbactam[a]	*Enterobacteriaceae*	≤ 2 to ≥ 64	≤ 8	16	≥ 32
Aztreonam	*Enterobacteriaceae*	≤ 1 to ≥ 32	≤ 4	8	≥ 16
	P. aeruginosa	≤ 2 to ≥ 64	≤ 8	16	≥ 32
Cefepime[b]	*Enterobacteriaceae*	≤ 1 to ≥ 64	≤ 2	4 - 8	≥ 16
	P. aeruginosa	≤ 2 to ≥ 64	≤ 8	-	≥ 16
Ceftazidime[c]	*Enterobacteriaceae*	≤ 1 to ≥ 32	≤ 4	8	≥ 16
	P. aeruginosa	≤ 2 to ≥ 64	≤ 8	-	≥ 16
Ceftriaxone[d]	*Enterobacteriaceae*	≤ 0.25 to ≥ 8	≤ 1	2	≥ 4
Ciprofloxacin[e]	*Enterobacteriaceae*	≤ 0.25 to ≥ 8	≤ 1	2	≥ 4
	P. aeruginosa				
Ertapenem	*Enterobacteriaceae*	≤ 0.125 to ≥ 4	≤0.5	1	≥ 2
Gentamycin	*Enterobacteriaceae*	≤ 1 to ≥ 32	≤ 4	8	≥ 16
	P. aeruginosa				
Meropenem[f]	*Enterobacteriaceae*	≤ 0.25 to ≥ 8	≤ 1	2	≥ 4
	P. aeruginosa	≤ 0.5 to ≥ 16	≤ 2	4	≥ 8
Piperacillin/Tazobactam[g]	*Enterobacteriaceae*	≤ 4 to ≥ 256	≤ 16	32-64	≥ 128
	P. aeruginosa				
	A. baumannii				
Tobramycin	*Enterobacteriaceae*	≤ 1 to ≥ 32	≤ 4	8	≥ 16
	P. aeruginosa				

[a] Truncated Range for ampicillin/sulbactam: PRO (4-64)
[b] Truncated Range for cefepime: PAE (2-32)
[c] Truncated Range for ceftazidime: CIT, ECO, ENT, KLE, PAE (2-32)
[d] Truncated Range for ceftriaxone: PRO, SMA (0.5-8)
[e] Truncated Range for ciprofloxacin: ENT (0.5-8)
[f] Truncated Range for meropenem: ENT, PAE (1-16)
[g] Truncated Range for piperacillin/tazobactam: PAE (8-256)

Table 5. Results Applicable to Phenotypic Resistance Tests

Resistance Phenotype Test	ID Target		
	SAU	SLU	CNS
Cefoxitin (MRSA)	Pos/Neg	Pos/Neg	Pos/Neg
Erythromycin/Clindamycin (MLSB)	N/A	Pos/Neg	Pos/Neg

Performing the Assay

The sample is collected from a positive blood culture using a sterile collection device. Blood culture samples should be tested within eight hours of the positive detection as determined by the continuous monitoring blood culture system. Samples may be stored at room temperature or the blood culture bottle can remain in the blood culture instrument until the sample is withdrawn for testing. The blood culture sample is vortexed and 0.5 mL is added to the sample vial. The test must be initiated within 15 minutes of placing the blood culture sample in the sample vial. The sample vial is inserted into the sample vial receptacle on the reagent cartridge, and the test cassette and reagent cartridge are placed into the Pheno instrument.

Test Results and Interpretation

Identification Assay. When the assay is complete, the Accelerate Pheno system displays the final results in a Patient Report or a Quality Control report. The "Identification Results" section lists all target groups tested and indicates which target group was detected. Target groups with positive and/or indeterminate results are printed at the top of the report in bold font. Identification results can be as follows: Positive (target group detected); Negative (target group not detected); and Indeterminate (result not defined, target group may or may not be present). An "invalid" result (a test for which too few cells are present in the control channel) is not specifically reported; for samples with invalid results the Patient Report will indicate "*No ID results reported; too few cells for analysis. Perform alternate testing method for identification and susceptibility results.*"

When the PhenoTest BC kit detects an organism present in a positive blood culture for which there is no target probe in the PhenoTest BC kit (off-panel organism), the Patient Report indicates negative results for all target probes and instructs the user to perform an alternate testing method.

For samples for which two or more target species are identified, the Patient Report will recommend that culture should be performed "*Recommend culture due to possibility of another organism being present.*"

The PhenoTest BC Kit does not provide speciation of *Streptococcus* spp. If the result indicates the presence of a *Streptococcus* species, and that additional testing should be performed to rule out *Streptococcus pneumoniae*. The following limitation is included in the device labeling:

> *Subculture is required for the identification of S. pneumoniae in cases of a positive Streptococcus spp. call.*

AST Assay. Antimicrobial susceptibility results are listed on the Patient Report for a single target species. Only one AST result will be reported for any culture; susceptibility testing for additional organisms (if present) should be performed using an alternate method from subcultured sample. The following limitation is included in the device labeling:

If an AST result is not provided by the Accelerate PhenoTest BC kit, susceptibility testing must be performed using an alternative method.

The AST results included in the PhenoTest BC report will indicate the MIC, the breakpoints, and interpretive categories for the drugs for which testing was performed. Results for resistance phenotypes (cefoxitin and MLSb) are reported as positive or negative.

Quality Control.

For the identification assay the Quality Control Report indicates an overall status of "passed" or "failed" and will list the identification results and pass/fail status for each tested species based on detection (positive or negative) and correct identification of the QC strain. For the AST assay the Quality Control report lists the QC range, MIC and pass/fail status for each tested antimicrobial agent. Pass/fail status is determined by the whether the MIC result falls within the QC range (pass) or outside of the QC range (fail).

Gram Stain Correlation

All identification results provided by the PhenoTest BC kit are intended to be interpreted in conjunction with results obtained from Gram stain of the positive blood culture bottle. Gram reaction (gram-positive or gram-negative) and cellular morphology (gram-positive cocci in clusters, pairs or chains, gram-negative rods) should be considered in the correlation with PhenoTest BC results. Gram stain results showing gram-negative cocco-bacilli may indicate the presence of *Acinetobacter baumannii*; however the cellular morphology is inconsistent or difficult to interpret for members of this genus. If the Gram stain results differ from the expected Gram stain morphology for the organism(s) identified by the PhenoTest BC kit, results should be confirmed with an alternate method. In addition, for any sample for which PhenoTest BC has indicated a monomicrobial result and for which the Gram stain of the positive blood culture shows multiple morphologies, results should be confirmed with an alternate method. All Patient Reports include the following footnote:

Identification results that are discordant with Gram stain should be confirmed with an alternate method.

Monomicrobial Call

The PhenoTest BC kit will report a monomicrobial call for samples for which a target organism is identified and for which there is no additional evidence of the presence of an additional organism (on- or off-panel). Results of all monomicrobial calls should be correlated with results from Gram stain of the positive blood culture bottle. Patient results for samples that are considered monomicrobial will include the following footnote:

Monomicrobial: sample positive for only one pathogen

J. Standard/Guidance Document Referenced:

- CLSI EP-05-A3, Evaluation of Precision Performance of Quantitative Measurement, Approved Guideline, 2004
- CLSI M100-S26, Performance Standards for Antimicrobial Susceptibility Testing, 2015
- CLSI M02-A12, Performance Standards for Antimicrobial Disk Susceptibility Testing, 2015
- CLSI EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, 2005

- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline, 2012
- CLSIM07-A10, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard, 2015
- EN 62304-2006, Medical Device Software – Software Life-Cycle Processes, 2006

K. Test Principle:

The Accelerate Pheno system uses an automated sample preparation and surface immobilization method to enable microscopy-based, single-cell analysis for identification (ID) and antimicrobial susceptibility testing (AST). Identification is accomplished via fluorescence *in situ* hybridization (FISH). Antimicrobial susceptibility testing uses microscopic observation of individual, live, growing bacterial cells in near real time (approximately every 10 minutes) in the presence of antimicrobial agents.

The Accelerate Pheno system employs automated sample and reagent pipetting, temperature controlled incubation, digital microscopy, image acquisition and analysis in an integrated and fully automated system.

L. Performance Characteristics:

1. Analytical performance:

   a. *Precision/Reproducibility:*
   Reproducibility studies of the Accelerate PhenoTest BC Kit for positive blood culture organism identification (ID) and antimicrobial susceptibility testing (AST) included evaluation of four replicates of the same positive blood culture sample on the same day at three testing sites for a total of 12 tests per blood culture sample. (Four replicates were initially tested to assure at least three valid results for each sample.) Samples were tested within eight hours of positivity. Primary probe targets utilized for reproducibility testing of the ID assay included representative species of each major organism group (*S. aureus, S. pneumoniae, E. faecium, E. coli, A. baumannii* and *C. albicans*). Additional species were tested in order to obtain the expected number of AST results (*K. pneumoniae*, *P. aeruginosa*, *Citrobacter* spp., *Enterobacter* spp., coagulase negative *Staphylococcus*, *Proteus* spp., and *S. marcescens,* and *E. faecalis*). A minimum of 90 data points were evaluated for each probe target.

   The reproducibility of the organism identification assay was assessed on a per probe basis across all study sites and within each study site. A minimum of nine ID results (three per site) was evaluated for each sample. The ID results were classified as "correct" or "not correct" by comparing the observed ID to the known ID for each test strain.

   Isolates that provided at least three ID results per site but which failed to produce at least three AST results per site were retested. All ID results were included in the analysis of probe performance. In order not to confound ID probe performance with overrepresentation of a single species, weighted percentages were calculated.

   For the identification assay, 11 of the 12 probe targets showed reproducibility > 95%. The *Enterobacter* probe (ENT) initially showed a reproducibility of 87.5% due to false negative results. Root cause analysis resulted in a post study imaging processing change; the original data was reevaluated with regression analysis. The resulting reproducibility was 93.2%

(Table 6).

Table 6. Summary of Reproducibility of Identification Assay Results:

Probe[a]	No. Detected/ No. Valid	No. Detected/ No.Valid	No. Detected/ No.Valid	Total Identified	Percent Identified	Weighted Percent Identified
	Site 34	Site 35	Site 36			
ABA	62/62	63/63	60/64	185/189	97.9%	98.7%
CAL	43/44	43/44	43/45	129/133	97.0%	96.7%
CIT[b]	17/17	30/32	30/32	77/81	95.1%	94.4%
ECO	55/55	51/52	55/55	161/162	99.4%	99.2%
EFM	51/53	55/56	49/54	155/163	95.1%	97.0%
EFS[b]	8/8	8/8	8/8	24/24	100.0%	100.0%
ENT[b]	19/24	21/23	20/24	60/71	84.5%[b]	87.5%[c]
KLE	46/48	46/48	44/48	136/144	94.4%	96.3%
PAE	43/43	48/48	48/48	139/139	100.0%	100.0%
SAU	131/135	138/140	136/137	405/412	98.3%	98.0%
SMA[b]	8/8	8/8	8/8	24/24	100.0%	100.0%
STR	38/39	38/38	35/35	111/112	99.1%	99.2%

[a] See Table 1 for abbreviation definitions

[b] Isolates of *Citrobacter* spp., *E. faecalis, Enterobacter* spp. and *S. marcescens* were evaluated in order to obtain sufficient results for AST Testing; total number of data points for each species <90.

[c] An Image processing change was implemented post study to correct a known issue. Regression analysis of the ENT probe produced a percent identified of 93.0% and a weighted percent identified score of 93.2%.

For AST, reproducibility for all antimicrobials was assessed by evaluating approximately 10 organisms with on-scale MIC values (a value within the assay's reportable range) for each antimicrobial reported by the assay. For Daptomycin and Vancomycin fewer than ten isolates were tested due to the lack of isolates with on-scale MICs for these antimicrobials; however, more than four replicates per isolate were tested to achieve 90 data points for those antimicrobials. AST reproducibility was determined from the total number of results that fell within 1 dilution (+/- one doubling dilution) of the mode results divided by the total number of results. On-scale AST performance for each antimicrobial agent was evaluated between sites and within each site. Both Best Case (assumes that off-scale results are within one dilution of the mode) and Worse Case (assumes that off-scale results are more than one dilution of the mode) performance was determined for each drug when the mode across all sites was on-scale for each drug. Worst case performance was not determined for the resistance phenotypes as this result is positive/negative with no off-scale results. Only those samples that met the required number of total results per site were used in the final calculations.

For the AST assay testing the reproducibility of 17 of the 18 antimicrobials and resistance phenotypes were acceptable with best case reproducibility of > 95%. However, erythromycin demonstrated best and worst case reproducibility of 93.6% which was considered acceptable (Table 7).

Indeterminate, false positive and invalid results were obtained during the course of the reproducibility study. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

Table 7. Summary of Reproducibility of AST Assay Results for all Antimicrobials and Resistance Phenotypes across All Sites

Antimicrobial	Organisms Tested	No. isolates	Best Case No. Within 1 ± Dil/ Total Tests (%)	Worst Case No. Within 1 ± Dil/ Total Tests (%)
Amikacin	*A. baumannii*	6	128/128 (100.0)	118/128 (92.2)
	P. aeruginosa	4		
	S. marcescens	1		
Ampicillin	*E. faecium*	8	93/93 (100.0)	88/93 (94.6)
Aztreonam	*C. freundii*	3	348/350 (99.4)	348/350 (99.4)
	K. pneumoniae	10		
	E. coli	11		
	P. aeruginosa	6		
	S. marcescens	1		
Ceftazidime	*C. freundii*	3	324/331 (100.0)	317/331 (95.8)
	K. pneumoniae	7		
	E. coli	11		
	P. aeruginosa	7		
	S. marcescens	1		
Ceftaroline	*S. aureus*	11	151/151/ (100.0)	146/151 (96.7)
Ciprofloxacin	*C. freundii*	1	136/139 (100.0)	133/139 (95.7)
	K. pneumoniae	2		
	E. coli	2		
	P. aeruginosa	5		
	S. marcescens	1		
	E. aerogenes	1		
Ceftriaxone	*C. freundii*	1	151/151 (100.0)	140/151 (92.7)
	K. pneumoniae	2		
	E. coli	8		
	S. marcescens	2		
Daptomycin	*S. aureus*	3	92/92 (100.0)	82/92 (89.1)
	S. haemolyticus	1		
	E. faecalis	1		
Erythromycin	*S. aureus*	10	117/125 (93.6)	117/125 (93.6)
Ertapenem	*C. freundii*	3	356/356 (100.0)	354/356 (99.4)
	K. pneumoniae	11		
	E. coli	12		
	S. marcescens	2		
	E. aerogenes	3		
Cefepime	*C. freundii*	1	158/162 (97.5)	152/162 (93.8)
	K. pneumoniae	1		
	E. coli	8		
	P. aeruginosa	2		
	S. marcescens	1		
	E. aerogenes	1		

Antimicrobial	Organisms Tested	No. isolates	Best Case No. Within 1 ± Dil/ Total Tests (%)	Worst Case No. Within 1 ± Dil/ Total Tests (%)
Gentamycin	*K. pneumoniae*	6	233/234 (99.6)	218/234 (93.2)
	E. coli	5		
	P. aeruginosa	6		
	S. marcescens	2		
	E. aerogenes	1		
Linezolid	*E. faecium*	10	350/355 (98.6)	350/355 (98.6)
	E. faecalis	2		
	S. aureus	17		
Meropenem	*P. aeruginosa*	7	105/106 (99.1)	104/106 (98.1)
	S. marcescens	2		
Amp/Sulbactam	*K. pneumoniae*	6	157/157 (100.0)	152/157 (96.8)
	E. coli	8		
Tobramycin	*K. pneumoniae*	2	102/104 (98.1)	101/104 (97.1)
	E. coli	2		
	P. aeruginosa	3		
	S. marcescens	2		
Pip/Tazo	*C. freundii*	2	215/222 (96.9)	205/222 (92.3)
	K. pneumoniae	4		
	E. coli	4		
	P. aeruginosa	7		
	S. marcescens	1		
	E. aerogenes	1		
Vancomycin	*E. faecium*	1	268/282 (95.0)	232/282 (82.3)
	S. aureus	7		
Resistance Phenotypes				
Cefoxitin	*S. aureus*	21	206/206 (100.0)	N/A[b]
MLSb	*S. aureus*[a]	21	217/222 (97.8)	N/A[b]

[a] Reproducibility was performed with 21 isolates of *S. aureus* that were MLSb positive and negative; however in the clinical study, major and minor errors occurred that could not be resolved; there is no claim for testing *S. aureus* for MLSb and results will be suppressed.
[b] Resistance phenotype, no worst case evaluation.

b. *Linearity/assay reportable range:*
Not Applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Three internal assay controls are included in each Accelerate PhenoTest BC kit and require no action by the user:

Universal Bacterial Probe (or Universal Eukaryotic Probe for Yeast). The universal bacterial probe binds to rRNA in all bacterial cells to signal that bacterial cells are present in the flowcells and to differentiate bacteria from debris. The universal eukaryotic probe binds to rRNA in all eukaryotic cells, including yeast, to signal that yeast cells are present in the flowcells and to differentiate yeast from debris. Colocalization of universal and target probe is required for identification of a target organism.

General Nucleic Acid Stain. A cell permeant nucleic acid fluorescent dye that binds to double-stranded DNA (dsDNA) is run in a separate flowcell channel as a DNA staining control. Data from this control is used to quantify the total number of microbial cells in a sample. Based on this result the remainder of the sample is diluted in order to load the desired number of cells per microscopic field of view for susceptibility testing.

Growth Control Channel. The growth control channel is used as a positive control for AST. It measures the number of healthy clones as well as the growth density and growth rate of healthy cells that are not exposed to any antibiotics. The channel consists of the sample and Mueller-Hinton Agar as the growth media for the cells as recommended by CLSI for susceptibility testing.

External Controls. Organisms used for QC testing of the PhenoTest BC kit are included in four panels (Table 8); multiple isolates are tested in a single run by loading the members of a single panel into specified wells in the reagent cartridge. QC organism panels can be rotated to ensure that each ID and AST channel is tested on a regular basis.

Table 8. Quality Control Organism Panels

<table>
<tr><th>Panel Name</th><th>Organisms</th><th>Strain</th></tr>
<tr><td rowspan="5">EPEES Panel (AST)</td><td>E. coli</td><td>ATCC 25922</td></tr>
<tr><td>E. coli</td><td>ATCC 35218</td></tr>
<tr><td>P. aeruginosa</td><td>ATCC 27853</td></tr>
<tr><td>E. faecalis</td><td>ATCC 29212</td></tr>
<tr><td>S. aureus</td><td>ATCC 29213</td></tr>
<tr><td colspan="3"></td></tr>
<tr><td rowspan="2">SES Panel (AST)</td><td>S. aureus</td><td>ATCC 43300</td></tr>
<tr><td>S. aureus</td><td>ATCC BAA-977</td></tr>
<tr><td colspan="3"></td></tr>
<tr><td rowspan="6">CASKS Panel (ID)</td><td>S. lugdunensis</td><td>ATCC 700328</td></tr>
<tr><td>K. pneumoniae</td><td>ATCC 700603</td></tr>
<tr><td>A. baumannii</td><td>ATCC 19606</td></tr>
<tr><td>C. freundii</td><td>ATCC 6879</td></tr>
<tr><td>C. glabrata</td><td>ATCC 2001</td></tr>
<tr><td>S. agalactiae</td><td>ATCC 12403</td></tr>
<tr><td colspan="3"></td></tr>
<tr><td rowspan="6">SPECS Panel (ID)</td><td>E. faecium</td><td>ATCC 19434</td></tr>
<tr><td>S. marcescens</td><td>ATCC 43862</td></tr>
<tr><td>E. aerogenes</td><td>ATCC 13048</td></tr>
<tr><td>P. vulgaris</td><td>ATCC 6380</td></tr>
<tr><td>C. albicans</td><td>ATCC 96268</td></tr>
<tr><td>S. pneumoniae</td><td>ATCC 49619</td></tr>
</table>

QC Reportable Range. The dilutions used for evaluation of QC strains are different than the reportable ranges used for patient isolates (Table 9). These ranges were added for the purpose of generating on-scale QC results; these ranges are not the same as those used for reporting patient samples from the PhenoTest BC kit.

Table 9. Reportable Ranges for QC organisms

QC Strain	Drug	QC Reportable Range (µg/mL)
E. coli ATCC 25922	Ceftriaxone	0.015 – 0.25
	Ceftazidime	0.03 - 1
P. aeruginosa ATCC 27853	Amikacin	0.5 - 8
	Aztreonam	1 – 16
	Cefepime	0.25 – 8
	Ciprofloxacin	0.12 – 2
	Ertapenem	1 – 16
	Gentamicin	0.25 – 4
	Meropenem	0.25 – 2
	Tobramycin	0.25 - 2
E. coli ATCC 35218	Ampicillin/Sulbactam	4 – 64
	Piperacillin/Tazobactam	0.25 - 4
E. faecalis ATCC 29212	Ampicillin	0.25 – 4
	Daptomycin	0.5 – 8
	Linezolid	0.5 – 8
	Vancomycin	0.5 - 8
S. aureus ATCC 29213	Cefoxitin	Negative*
	Ceftaroline	0.25 – 1
	Erythromycin	0.25 – 2
	MLSb	Negative*
S. aureus ATCC 4330	Cefoxitin	Positive*
S. aureus ATCC BAA-977	MLSb	Positive*

*Qualitative Assay, No reportable range

Quality Control Testing. Quality Control was run each day during the clinical and analytical studies. To monitor system performance during the clinical study, QC organism panels were rotated to ensure that each ID and AST channel was tested on a regular basis. The reference laboratory performed QC each day of testing with appropriate organisms for the reference methods. QC results are shown below for the Identification assay (Table 10) and the AST assay (Table 11). Quality control results were acceptable.

Table 10. QC Results Identification Assay, Clinical Study

QC Organism	No. Tested	No. Detected	No. Not Detected	Percent Detected
A. baumannii ATCC 19606	406	398	8	98.0
C. albicans ATCC96268	390	385	5	98.7
C. glabrata ATCC 2001	406	388	18	95.6
C. freundii ATCC6879	406	389	17	95.8
E. aerogenes ATCC 13048	390	388	2	99.5
E. faecalis ATCC 29212	578	567	11	98.1
E. faecium ATCC 19434	390	382	8	98.0
E. coli ATCC 25922	578	562	16	97.2
K. pneumoniae ATCC 700603	406	400	6	98.5
P. vulgaris ATCC 6380	390	382	8	98.0
P. aeruginosa ATCC 27853	578	567	11	98.1

QC Organism	No. Tested	No. Detected	No. Not Detected	Percent Detected
S. marcescens ATCC 43862	390	388	2	99.5
S. aureus ATCC 43300	354	352	2	99.4
S. lugdunensis ATCC 70028	406	400	6	98.5
S. agalactiae ATCC 12403	406	394	12	97.0
S. pneumoniae ATCC 49619	390	383	7	98.2

Table 11. QC Results AST Assay, Clinical Study

Drug	QC Organism	Expected Range (µg/mL)	Reference		PhenoTest BC	
			No. Tested	No. in Range (%)	No. Tested	No. in Range (%)
Amikacin	*P. aeruginosa* ATCC 27853	1 - 4	67	67 (100.0)	284	284 (100.0)
Ampicillin	*E. faecalis* ATCC 29212	0.5 - 2	64	64 (100.0)	278	277 (99.6)
Ampicillin/ Sulbactam	*E. coli* ATCC 35218	8/4 – 32/16	67	66 (98.5)	286	285 (99.7)
Aztreonam	*P. aeruginosa* ATCC 27853	2 - 8	66	66 (100.0)	285	285 (100.0)
Cefepime	*P. aeruginosa* ATCC 27853	0.5 - 4	67	67 (100.0)	285	284 (99.7)
Ceftazidime	*E. coli* ATCC 25922	0.06 – 0.5	69	69 (100.0)	286	286 (100.0)
Ceftaroline	*E. faecalis* ATCC 29212	0.25 - 2	64	64 (100.0)	(100)	279 (100.0)
Ciprofloxacin	*P. aeruginosa* ATCC 27853	0.25 - 1	67	67 (100.0)	285	285 (100.0)
Ceftriaxone	*E. coli* ATCC 25922	0.03 – 0.125	64	64 (100.0)	286	286 (100.0)
Daptomycin	E. faecalis ATCC 29212	1 - 4	64	64 (100.0)	279	279 (100.0)
Erythromycin	*S. aureus* ATCC 29213	0.25 - 1	61	61 (100.0)	284	282 (99.3)
Ertapenem	*P. aeruginosa* ATCC 27853	2 - 8	67	67 (100.0)	285	285 (100.0)
Gentamycin	*P. aeruginosa* ATCC 27853	0.5 - 2	67	67 (100.0)	285	285 (100.0)
Linezolid	*E. faecalis* ATCC 29212	1 - 4	64	64 (100.0)	279	279 (100.0)
Meropenem	*P. aeruginosa* ATCC 27853	0.25 - 1	66	66 (100.0)	284	282 (99.3)
Piperacillin/ Tazobactam	*E. coli* ATCC 35218	0.5/4 – 2/4	70	69 (98.6)	285	285 (100.0)
Tobramycin	*P. aeruginosa* ATCC 27853	0.25 - 1	69	69 (100.0)	285	283 (99.3)
Vancomycin	*E. faecalis* ATCC 29212	1 - 4	64	64 (100.0)	279	279 (100.0)
Cefoxitin	*S. aureus* ATCC 29213	Neg	62	62 (100.0)	285	282 (98.9)

Drug	QC Organism	Expected Range (µg/mL)	Reference		PhenoTest BC	
			No. Tested	No. in Range (%)	No. Tested	No. in Range (%)
	S. aureus ATCC 43300	Pos	61	61 (100.0)	172	172 (100.0)
MLSb	*S. aureus* ATCC 29213	Neg	62	62 (100.0)	285	282 (99.0)
	S. aureus ATCC BAA-977	Pos	64	64 (100.0)	174	174 (100.0)

d. *Growth and Detection Study*
The Growth and Detection study was performed to evaluate two processes: 1) the validation of the inoculum concentration for seeded blood cultures and 2) to assess the equivalence and repeatability of identification and AST results at the time of positivity (t=0) and at eight hours post-positivity (t=8).

Validation of inoculum concentration. This study was performed to demonstrate that blood culture bottles inoculated with various concentrations of a specific organism and incubated until positivity in a continuous monitoring blood culture instrument showed essentially equivalent organism concentrations at the time of positivity, regardless of the starting inoculum concentration.

For each of three representative organisms (*S. aureus, E. coli* and *C. albicans*) a standardized suspension was prepared and serially diluted. Each of three dilutions was inoculated into three blood culture bottles containing the appropriate volume of human blood. Bottles were incubated until positivity on the blood culture instrument. The organism concentration for each bottle was determined using standard plate counts and an aliquot of each bottle was tested with the PhenoTest BC Kit.

Results, Inoculum validation study. The intra-organism plate count averages at positivity were generally within one log across positive blood culture samples despite seeding concentrations that spanned four logs; the concentration of organisms was within the expected range of organism concentration in positive patient blood cultures at bottle ring. All positive blood culture bottles tested at t=0 produced the expected positive ID results for each organism (Table 12). Results of this study informed the seeding concentration for analytical studies and seeding studies.

Table 12. Spiking Concentration, Concentration at Positivity and Identification Results

Species/Isolate	Spiking Concentration	Mean Concentration at Positivity	No. Identified/No Tested (%)
***S. aureus* ATCC 29213**	$1.5 X 10^3 – 3.0 X 10^3$	$9.4 X 10^6$	3/3 (100.0)
	$1.5 X 10^1 – 3.0 X 10^1$	$1.0 X 10^7$	3/3 (100.0)
	$1.5 X 10^{-1} – 3.0 X 10^{-1}$	$5.5 X 10^6$	5/5 (100.0)
***E. coli* ATCC 25922**	$1.5 X 10^3 – 3.0 X 10^3$	$5.1 X 10^8$	3/3 (100.0)
	$1.5 X 10^1 – 3.0 X 10^1$	$1.7 X 10^9$	3/3 (100.0)
	$1.5 X 10^{-1} – 3.0 X 10^{-1}$	$9.6 X 10^7$	3/3 (100.0)
***C. albicans* ATCC 96268**	$7.8 -40 X 10^3$	$1.7 X 10^7$	3/3 (100.0)
	$7.8 -40 X 10^1$	$4.3 X 10^6$	4/4 (100.0)
	$7.8 -40 X 10^{-1}$	$4.7 X 10^6$	4/4 (100.0)

Growth and Detection. The performance of the PhenoTest BC system was evaluated using seeded blood cultures prepared with 21 on-panel organisms and one off-panel organism selected to evaluate each of the 16 ID assays and 20 AST and phenotypic resistance determinants included in the PhenoTest BC Kit. Each isolate was spiked into BACTEC blood culture bottles containing the recommended quantity of human blood in concentrations equivalent to those determined in the inoculum validation study. Tests were performed in triplicate. The blood culture bottles were seeded, placed in the BACTEC blood culture instrument and incubated until positivity. The blood cultures were then tested with the PhenoTest BC kit at the time of positivity (t=0), placed back into the blood culture instrument and retested eight hours after positivity (t=8). The performance was evaluated by comparing the results to the expected result based on the known characteristics (ID and AST) of the spiked organisms. The concentration of organism in the tested blood cultures was determined at both the t=0 and t=8 time points using standard plate counts.

Results Growth and Detection Results, ID Assay. Samples tested at t=8 demonstrated organism concentrations approximately 1 log higher than samples tested at t=0 for the majority of organisms. *S. lugdunensis, P. mirabilis* and *C. glabrata* had organism concentrations that differed between the two time points at approximately 1.5 logs, 2 log and < 1 log, respectively (Table 13). Correct identifications were obtained for all samples at both t=0 and t=8 time points, validating the sponsor's claim for testing positive blood culture bottles within 8 hours of bottle ring

Table 13. Growth and Detection Study, ID Assay

Organism	t=0 No. Identified/ Total Tested (%)	t=8 No. Identified/ Total Tested (%)	Concentration Difference t=0 and t=8
S. aureus *S. epidermidis* *E. faecalis* *E. faecium* *S. gallolyticus* *S. mitis*	21/21 (100.0)	21/21 (100.0)	1 log
S. lugdunensis	3/3 (100.0)	3/3 (100.0)	~1.5 log
C. freundii *C. koseri*	34/34 (100.0)	34/34 (100.0)	1 log

Organism	t=0 No. Identified/ Total Tested (%)	t=8 No. Identified/ Total Tested (%)	Concentration Difference t=0 and t=8
E. aerogenes *E. cloacae* *E. coli* *K. oxytoca* *K. pneumoniae* *P. vulgaris* *S. marcescens* *P. aeruginosa* *A. baumannii*			
P. mirabilis	3/3 (100.0)	4/4 (100.0)	~2 log
C. albicans *C. glabrata*	4/4 (100.0)	3/3 (100.0)	< 1 log

Results Growth and Detection, AST Assay. In the Growth and Detection Study the AST results were evaluated for two parameters: 1) Consistency and equivalence of MIC values reported for all antimicrobials and organisms at both time points t=0 and t=8, and 2) Essential agreement and categorical agreement of results obtained at t=0 and t=8 as compared to the reference method.

For parameter 1, The mode values obtained for samples tested at t=0 and t=8 were within one ± doubling dilution for all isolates tested with all antimicrobials except *P. mirabilis* with meropenem and *C. freundii* with piperacillin-tazobactam. On repeat, the mode of the *P. mirabilis* MIC values with meropenem was acceptable; results of *C. freundii* with piperacillin-tazobactam were not resolved. However, the overall repeatability of all MIC values was 421/429 (98%) indicating that the PhenoTest BC Kit will provide reproducible AST results with positive blood culture bottles tested between 0 and 8 hours post bottle ring.

For parameter 2, all results with all organisms tested with the following antimicrobials showed essential agreement and categorical agreement ≥ 89.9% as compared to results obtained with the reference method: amikacin, ampicillin, ampicillin-sulbactam, ceftaroline, ciprofloxacin, daptomycin, erythromycin, meropenem and vancomycin. Cefoxitin and MLSb showed category agreement of 100% with the reference method. Comparative results with the reference method obtained with the remaining antimicrobials reflected the lower EA and/or CA obtained for those antimicrobials in the clinical study; these performance issues were subsequently addressed through limitations. Results of AST testing at t=0 and t=8 are shown in Table 14:

Table 14. Growth and Detection Study, AST Assay, EA and CA as Compared to the Reference Method at t=0 and t=8

Antimicrobial	Organisms	t=0		t=8	
		EA (%EA)	CA (%CA)	EA (%EA)	CA (%CA)
Amikacin	*Enterobacteriaceae* *A. baumannii* *P. aeruginosa*	36/36 (100.0)	36/36 (100.0)	36/36 (100.0)	36/36 (100.0)
Ampicillin	*Enterococcus* spp.	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)
Ampicillin-Sulbactam	*Enterobacteriaceae*	15/15 (100.0)	14/15 (93.3)	15/15 (100.0)	14/15 (93.3)
Aztreonam	*Enterobacteriaceae* *P. aeruginosa*	30/33 (90.0)	25/33 (75.7)[a]	28/33 (84.4)	25/33 (75.7)[a]
Cefepime	*Enterobacteriaceae* *P. aeruginosa*	29/33 (87.9)	25/33 (75.8)[a]	29/33 (87.9)	26/33 (78.8)[a]
Ceftaroline	*S. aureus*	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)
Ceftazidime	*Enterobacteriaceae* *P. aeruginosa*	27/33 (81.8)	28/33 (84.8)[a]	27/33 (81.8)	27/33 (81.8)[a]
Ceftriaxone	*Enterobacteriaceae*	26/30 (86.7)	24/30 (80.0)[a]	27/30 (90.0)	24/30 (80.0)[a]
Ciprofloxacin	*Enterobacteriaceae* *P. aeruginosa*	33/33 (100.0)	33/33 (100.0)	33/33 (100.0)	33/33 (100.0)
Daptomycin	*Staphylococcus* spp. *Enterococcus* spp.	15/15 (100.0)	15/15 (100.0)	15/15 (100.0)	15/15 (100.0)
Ertapenem	*Enterobacteriaceae*	24/30 (80.0)	21/30 (70.0)[a]	24/30 (80.0)	21/30 (70.0)[a]
Erythromycin	*S. aureus*	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)
Gentamicin	*Enterobacteriaceae* *P. aeruginosa*	332/33 (97.0)	26/33 (78.8)[a]	33/33 (100.0)	28/33 (84.8)[a]
Linezolid	*S. aureus*	9/9 (100.0)	6/9 (66.7)[b]	9/9 (100.0)	6/9 (66.7)[b]
Meropenem	*Enterobacteriaceae* *P. aeruginosa*	32/33 (97.0)	32/33 (97.0)	32/33 (97.0)	32/33 (97.0)
Piperacillin-tazobactam	*Enterobacteriaceae* *P. aeruginosa*	32/36 (88.9)	30/36 (83.3)[a]	27/36 (75.9)	27/36 (75.0)[a]
Tobramycin	*Enterobacteriaceae* *P. aeruginosa*	25/33 (75.8)	33/33 (100.0)	26/33 (78.8)	29/33 (87.9)
Vancomycin	*Staphylococcus* spp. *Enterococcus* spp.	15/15 (100.0)	15/15 (100.0)	15/15 (100.0)	15/15 (100.0)
Cefoxitin	*Staphylococcus* spp.	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)
MLSb	*Staphylococcus* spp.	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)

[a] Low category agreement due to a majority of minor errors
[b] Low number of isolates tested

Indeterminate, false positive and invalid results were obtained during the course of the growth and detection study. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

e. *Cross Contamination/Carry Over*
Evaluation of cross contamination and carryover was performed as a component of the Growth and Detection studies. In general t=0 and t=8 samples containing different organisms were run in alternating fashion to evaluate any potential carryover from samples with higher organism loads to samples with lower organism concentrations.

Results Cross Contamination/Carry-over Study. None of the alternating organism species and concentration combinations tested on the same instrument demonstrated any evidence of cross contamination.

f. *Analytical Inclusivity*
The intent of the inclusivity study was to demonstrate the ability of the PhenoTest BC Kit to detect and identify on-panel species in positive blood culture samples. The Inclusivity (Analytical Specificity) of the PhenoTest BC Kit was evaluated by testing three strains of each of the on-panel target species. (Additional well-characterized strains were evaluated as challenge organisms for the AST assay; results obtained with the PhenoTest BC Kit with these organisms are included in the analysis of the Identification assay performance.) A standardized suspension of each organism was inoculated into blood culture bottles containing the appropriate volume of human blood and incubated until determined positive by the blood culture instrument. Testing with the PhenoTest BC Kit was then performed within approximately 1 hour. All isolates included in the Inclusivity Study are listed in Tables 15 -17 were detected and correctly identified by the PhenoTest BC Kit.

Table 15. Inclusivity Study: Gram-Positive Isolates Tested for Each Probe Target

Probe	Species	Strain
SAU	*Staphylococcus aureus*	IHMA 166917, ATCC 10832, ATCC 29213
CNS	*Staphylococcus epidermidis*	JMI 365, JMI 154
	Staphylococcus haemolyticus	ATCC 29970, JMI 4510, JMI 18615*
	Staphylococcus lugdunensis	ATCC 700328, JMI 364, JMI 367
	Staphylococcus capitis	ATCC 27840, ATCC 35661, JMI 794
	Staphylococcus hominis	ATCC 27844*, JMI 16176, JMI 16434
SLU	*Staphylococcus lugdunensis*	ATCC 700328, JMI 364, JMI 367
EFS	*Enterococcus faecalis*	IHMA 850419, ATCC 19433, ATCC 29212
EFM	*Enterococcus faecium*	IHMA 743951, ATCC 19434, BEI N28976
STR	*Streptococcus agalactiae* (serotype Ia)	ATCC BAA 1177
	Streptococcus agalactiae (serotype III)	ATCC 12403
	Streptococcus agalactiae (serotype V)	ATCC BAA 611
	Streptococcus gallolyticus	ATCC 43143, ATCC 51879, ATCC 700065
	Streptococcus mitis	IHMA 233226, HM 262, NR 34818
	Streptococcus pneumoniae (serotype 19A)	BEI H145
	Streptococcus pneumoniae (serotype 11A)	BEI N19172
	Streptococcus pneumoniae (serotype 6B)	BEI N19213*

* Indeterminate results obtained for additional probes

Table 16. Inclusivity Study: Gram-Negative Isolates Tested for Each Probe Target

Probe	Species	Strain
ABA	*Acinetobacter baumannii*	IHMA 770910, ATCC 19606, BEI N17785
CIT	*Citrobacter freundii*	IHMA 617760, JMI 1333*, ATCC 6879*
	Citrobacter koseri	IHMA 802066*, JMI 192*, JMI 631*
ENT	*Enterobacter aerogenes*	IHMA 832805, ATCC 13048, JMI 897
	Enterobacter cloacae	IHMA 814234, ATCC 13047, ATCC 35030
ECO	*Escherichia coli*	IHMA 511379, ATCC 11775, ATCC 25922
KLE	*Klebsiella oxytoca*	IHMA 501913, ATCC 700324, BEI H622
	Klebsiella pneumoniae	IHMA 875649, ATCC 4972, ATCC 700603
PRO	*Proteus mirabilis*	IHMA 1032951, BEI H752, JMI 148
	Proteus vulgaris	IHMA 451082, ATCC 6380, JMI 5687
PAE	*Pseudomonas aeruginosa*	IHMA 761927, ATCC 10145, ATCC 27853
SAE	*Serratia marcescens*	IHMA 946153, ATCC 43862, JMI 39780

* Indeterminate results obtained for additional probes

Table 17. Inclusivity Study: Yeast Isolates Tested for Each Probe Target

Probe	Species	Strain
CAL	*Candida albicans*	ATCC 96268, ATCC 11651, JMI 10981
CGL	*Candida glabrata*	JMI 52276, JMI 28365, JMI 21400

g. *Analytical Specificity (Exclusivity)*

The Exclusivity (Analytical Cross-Reactivity) of the PhenoTest BC Kit was evaluated by testing individual representative strains of on- and off-panel species. On-panel isolates were evaluated to determine if they would cross react with probes other than the expected probe. A standardized suspension of each organism was inoculated into blood culture bottles containing the appropriate volume of human blood. Testing with the PhenoTest BC Kit was performed 7.5 to 9 hours post positivity.

The exclusivity test panel for each probe cocktail was designed to challenge the specificity of the probe cocktail with species predicted to have close sequence homology to the target.

Tables 18 and 19 list the species that were determined to be non-cross reactive by analytical testing. Some organisms gave indeterminate results for certain target probes; indeterminate results are not conclusively positive or negative.

Table 18. Organisms Determined to be Non-Cross Reactive by Analytical Testing

Gram-Positive		
Bacillus cereus	*Lactococcus lactis*	*Staphylococcus felis*[a]
Bacillus subtilis	*Macrococcus caseolyticus*[a]	*Streptococcus constellatus*
Clostridium perfringens	*Macrococcus equipercicus*[a]	*Streptococcus gordonii*
Corynebacterium amycolatum[c]	*Micrococcus luteus*	*Streptococcus intermedius*[b]
Corynebacterium jeikeium[a]	*Mycobacterium avium*	*Streptococcus thoraltensis*
Corynebacterium striatum	*Mycobacterium intracellulare*	*Streptococcus vestibularis*
Enterococcus saccharolyticus	*Mycobacterium smegmatis*	*Vagococcus fluvialis*

Granulicatella adiacens	*Nocardia nova*[d]	
Gram-Negative		
Achromobacter xylosoxidans	*Citrobacter sedlakii*	*Providencia rettgeri*
Acinetobacter calcoaceticus	*Dickeya chrysanthemi*	*Pseudomonas luteola*
Acinetobacter johnsonii	*Dickeya paradisiaca*	*Pseudomonas putida*
Acinetobacter lwoffii	*Enterobacter ludwigii*	*Pseudomonas stutzeri*
Acinetobacter radioresistens	*Escherichia albertii*	*Ralstonia pickettii*
Acinetobacter schindleri	*Escherichia blattae*	*Raoultella planticola*
Aeromonas hydrophila	*Moraxella catarrhalis*	*Shigella flexneri*
Bacteroides fragilis	*Morganella morganii*	*Stenotrophomonas maltophilia*
Bordetella bronchiseptica	*Neisseria meningitidis*	*Yersinia enterocolitica*
Bordetella parapertussis[e]	*Plesiomonas shigelloides*	*Yersinia pestis*[e]
Brenneria quercina	*Proteus hauseri*	*Yersinia pseudotuberculosis*
Citrobacter braakii		

[a] Gave indeterminate results with CNS probe
[b] Different replicates gave indeterminate results with CNS and SAU probe
[c] Different replicates gave indeterminate results with CNS, SLU and SAU probes
[d] Gave indeterminate results with CAL probe
[e] Gave indeterminate result with SLU probe

Table 19. Cross Reacting Organisms as Determined by Analytical Testing

Probe	Cross Reactive Organisms	
Gram-Positive		
S. aureus **(SAU)**	*Candida parapsilosis*	*Streptococcus pneumoniae*
	Pseudomonas nitroreducens	*Staphylococcus hominis*
	Candida parapsilosis	*Pantoea agglomerans*
Coagulase negative *Staphylococcus* (CNS)	*Acinetobacter haemolyticus*	*Staphylococcus equorum*
	Acinetobacter ursingii	*Staphylococcus kloosii*
	Candida krusei	*Staphylococcus lentus*
	Candida parapsilosis	*Staphylococcus pasteuri*
	Candida tropicalis	*Staphylococcus saprophyticus*
	Enterobacter hormaechei	*Staphylococcus schleiferi*
	Enterococcus canis	*Staphylococcus succinus*
	*Enterococcus dispar**	*Staphylococcus xylosus*
	Mycobacterium scrofulaceum	*Streptococcus anginosus*
	Nocardia farcinica	*Streptococcus mutans*
	Staphylococcus auricularis	*Streptococcus pneumoniae**
	Staphylococcus caprae	*Streptococcus sanguinis*
	*Staphylococcus cohnii**	
E. faecium **(EFM)**	*Enterobacter aerogenes*	*Staphylococcus succinus*
	Enterococcus gallinarum	*Streptococcus pneumoniae**
	Enterococus durans	
S. lugdunensis **(SLU)**	*Nocardia farcinica*	*Streptococcus pneumoniae*
Streptococcus **spp. (STR)**	*Candida krusei**	*Streptococcus parasanguinis*
	Staphylococcus sciuri	*Streptococcus pseudopneumoniae*
	*Streptococcus constellatus**	*Streptococcus pyogenes*
	Streptococcus mutans	

Gram-Negative		
***A. baumannii* (ABA)**	*Acinetobacter pittii* (*A.* genomospecies 3)	
***P. aeruginosa* (PAE)**	*Burkholderia cepacia*	*Shigella dysenteriae*
	Enterobacter cloacae	*Staphylococcus sciuri*
	Enterococcus casseliflavus	
***Citrobacter* spp. (CIT)**	*Citrobacter gillenii*	*Escherichia hermannii*
	*Citrobacter murliniae**	*Pseudomonas fulva**
	*Citrobacter youngae**	
***Enterobacter* spp. (ENT)**	*Enterobacter asburiae*	*Enterobacter mori*
	Enterobacter hormaechei	*Enterobacter nimipressuralis*
***Escherichia coli* (ECO)**	*Escherichia fergusonii*	*Shigella dysenteriae*
	*Pseudomonas fulva**	*Shigella sonnei*
	Shigella boydii	
***Klebsiella* spp. (KLE)**	*Brenneria goodwinii*	*Escherichia hermannii*
	Brenneria nigrifluens	*Escherichia vulneris*
	Brenneria salicis	*Klebsiella ozaenae*
	Cronobacter sakazakii	*Klebsiella variicola**
	Enterobacter kobei	*Pantoea agglomerans*
***Proteus* spp. (PRO)**	*Proteus penneri*	
***Serratia marcescens* (SMA)**	*Brenneria nigrifluens*	*Streptococcus parasanguinis*
	Pseudomonas oryzihabitans	
***Candida* spp.**		
***Candida albicans* (CAL)**	*Streptococcus pyogenes*	*S. capitus*
	P. aeruginosa	
***Candida glabrata* (PAE)**	*Acinetobacter haemolyticus*	*Salmonella enterica*
	Brenneria goodwinii	*Streptococcus pyogenes*
	Enterococcus casseliflavus	

* Indeterminate results obtained for additional probes

In silico analysis was performed to assess potential cross reactivity with target probes for organisms that were not available or did not grow in blood culture media or for which valid PhenoTest BC Kit results were not available due to an insufficient cell concentration. To assess any potential cross reactivity the 16S rRNA gene sequences of the organisms were compared to the sequences of the probe targets in a sequence database. Table 20 lists additional organisms that were predicted to cross react with the target probe sequences based on in silico analysis. The following organisms were not tested for cross reactivity by either analytical or in silico analysis: *Bordetella pertussis, Brenneria nigrifluens, Brenneria salicis (Erwinia salicis), Nocardia abscessus* and *Vibrio parahemolyticus.*

Table 20. Organisms Predicted to Cross React with Target Probes by *In Silico* Analysis[a, b]

Organism	Probe Which May be Positive Based on *in silico* Prediction
Acinetobacter nosocomialis (aka A. genomospecies 13)	ABA
Citrobacter braakii	CIT
Citrobacter koseri	CIT
Cronobacter sakazakii	KLE
Enterococcus canis	EFM
Enterococcus saccharolyticus	EFM
Morganella morganii	SMA
Streptococcus gordonii	STR
Streptococcus pseudopneumoniae	STR
Streptococcus sanguiinis	STR
Streptococcus vestibularis	STR

[a] *In silico analysis did not consistently predict results obtained in analytical testing*
[b] Inclusivity could not be predicted via *in silico* analysis for *Klebsiella oxytoca* (PPA of clinical study 90.0%) and *Proteus vulgaris* (PPA of clinical study 100%)

Indeterminate, false positive and invalid results were obtained during the course of the inclusivity and exclusivity studies. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

h. *LoD* (Limit of Detection)
The objective of the LoD study was to determine the lowest concentration of organism in blood culture bottles that could be reliably detected and identified (>95% of the time). The level of detection of the Accelerate PhenoTest BC Kit was determined for all 16 FISH probes by inoculating blood culture bottles containing 10mL human donor blood with representative strains of each target organism or group of organisms at levels below the theoretical limit of detection (LoD) of the assay and below the expected concentration at positivity. The bottles were incubated in an automated blood culture instrument for a predetermined period and removed for analysis with the Accelerate PhenoTest BC assay prior to positivity detection. Tests were repeated and incubated for a longer period of time if 95% positivity was not achieved. Colony counts were performed to determine the organism concentration at the time of testing. Additional blood culture bottles that were inoculated at the same time as those for testing with the PhenoTest BC Kit were used to determine the duration of incubation in the blood culture instrument that would result in a positive detection. The results of the study indicate that in most cases the LoD is at or below the concentration of organisms present when blood cultures are determined to be positive by the continuous monitoring blood culture system (as determined in the Growth and Detection study summarized above). The average LoD for gram-negative target bacteria, gram-positive bacteria and *Candida* sp. was 4×10^8, 5×10^8 and 2×10^6, respectively. The results of the LoD studies are summarized in Tables 21 – 23 below.

Table 21. Analytical Sensitivity (LoD) of Gram-Negative Species

Probe	Species	Strain	Time (hours)		Concentration at Testing (CFU/mL)	No. Positive/No. Tested (%)
			Time To Positive[a]	Incubation Period[b]		
ABA	*A. baumannii*	ATCC 19606	6.2	4.8	1.51×10^{8}	22/22 (100.0)
		IHMA 758265	6.2	4.8	5.27×10^{8}	22/22 (100.0)
CIT	*C. freundii*	IHMA 1029875	4.9	4.5	2.20×10^{8}	5/22 (22.7)
			4.9	5.0	4.67×10^{8}	22/22 (100.0)
	C. koseri	IHMA 1003484	4.4	4.3	3.20×10^{8}	21/22 (95.5)
ECO	*E. coli*	ATCC 25922	4.2	4.0	1.15×10^{8}	18/18 (100.0)
			4.7	4.0	9.00×10^{7}	22/22 (100.0)
		IHMA 752961	4.2	4.0	7.40×10^{7}	22/22 (100.0)
ENT	*E. aerogenes*	IHMA 1006490	6.1	5.3	1.67×10^{7}	1/22 (4.5)
			4.6	5.8	1.59×10^{9}	21/22 (95.5)
		IHMA 832805	4.2	5.8	2.01×10^{9}	22/22 (100.0)
KLE	*K. oxytoca*	IHMA 1000393	5.4	4.8	1.14×10^{8}	11/22 (50.0)
			4.6	6.0	2.67×10^{8}	22/22 (100.0)
	K. pneumoniae	ATCC 700603	4.5	4.8	5.33×10^{8}	13/22 (59.1)
			4.4	5.5	8.13×10^{8}	22/22 (100.0)
			4.6	6.0	3.09×10^{9}	20/22 (90.9)
PAE	*P. aeruginosa*	ATCC 27853	5.2	4.3	7.73×10^{7}	21/22 (95.5)
		IHMA 1004320	5.2	4.5	9.33×10^{8}	20/22 (90.9)
			6.4	4.3	7.60×10^{7}	22/22 (100.0)
PRO	*P. mirabilis*	IHMA 1003471	5.5	5.3	2.26×10^{7}	22/22 (100.0)
	P. vulgaris	IHMA 1008418	6.2	4.5	8.93×10^{7}	21/21 (100.0)
SMA	*S. marcescens*	IHMA 1005957	5.3	4.0	1.30×10^{7}	2/23 (8.7)
			4.8	4.8	2.77×10^{8}	22/22 (100.0)
		IHMA 946153	4.6	4.0	$2.65 \text{ X } 10^{7}$	12/23 (52.2)
			4.8	4.8	1.08×10^{8}	22/22 (100.0)

[a] Duration of incubation at the time of positivity as detected by the continuously monitored blood culture system

[b] Duration of incubation in the continuous monitoring blood culture system prior to testing with the PhenoTest BC Kit.

Table 22. Analytical Sensitivity (LoD) for Gram-Positive Species

Probe	Species	Strain	Time (hours)		Concentration (CFU/mL)	No. Positive/No. Tested (%)
			Time To Positive[a]	Incubation Period[b]		
CNS	*S. epidermidis*	ATCC 14990	7.1	4.8	3.75×10^{5}	0/23 (0.0)
			6.9	6.0	2.84×10^{6}	21/22 (95.5)
	S. haemolyticus	IHMA 1056877	7.6	5.5	2.80×10^{6}	4/22 (18.2)
			6.5	6.4	3.27×10^{7}	21/21 (100.0)
EFM	*E. faecium*	IHMA	5.4	4.8	3.37×10^{7}	20/20 (100.0)

Probe	Species	Strain	Time (hours)		Concentration (CFU/mL)	No. Positive/No. Tested (%)
			Time To Positive[a]	Incubation Period[b]		
		148266				
		IHMA 824486	5.6	5.3	1.80 x 10^8	22/22 (100.0)
EFS	*E. faecalis*	IHMA 850419	4.8	6.0	4.00 x 10^8	21/22 (95.5)
		IHMA 1001973	4.2	6.0	8.33 x 10^8	22/22 (100.0)
SAU	*S. aureus*	ATCC 29213	5.1	4.8	3.73 x 10^6	22/22 (100.0)
		IHMA 612311	5.0	4.8	1.51 x 10^7	22/22 (100.0)
SLU/CNS	*S. lugdunensis*	IHMA 845545	5.1	5.3	3.66 x 10^7	22/22 (100.0)
		IHMA 867361	7.3	4.8	3.49 x 10^7	21/21 (100.0)
STR	*S. agalactiae*	IHMA 753220	3.9	6.0	1.45 x 10^9	2/22 (9.1)[c]
			4.3	5.0	1.19 x 10^9	18/22 (81.8)[c]
	S. gallolyticus	IHMA 878329	4.3	4.3	2.29 x 10^8	22/22 (100.0)
	S. pneumoniae	ATCC 49619	5.0	5.0	4.87 x 10^9	17/22 (77.3)
			5.8	6.5	3.81 x 10^9	21/21 (100.0)

[a] Duration of incubation at the time of positivity as detected by the continuous monitoring blood culture system
[b] Duration of incubation in the continuous monitoring blood culture system prior to testing with the PhenoTest BC Kit.
[c] The LoD for *S. agalactiae* was not established.

Table 23. Analytical Sensitivity (LoD) for *Candida* spp.

Probe	Species	Strain	Time (hours)		Concentration (CFU/mL)	No. Positive/No. Tested (%)
			Time To Positive[a]	Incubation Period[b]		
CAL	*C. albicans*	ATCC 11651	11.3	11.3	1.93 x 10^6	22/22 (100.0)
		IHMA 693236	7.3	7.3	2.97 x 10^6	20/21 (95.2)
CGL	*C. glabrata*	ATCC 2001	7.0	7.0	4.07 x 10^6	22/22 (100.0)
		JMI 29546	7.6	4.4	6.13 x 10^5	21/22 (95.5)

[a] Duration of incubation at the time of positivity as detected by the continuous monitoring blood culture system
[b] Duration of incubation in the continuous monitoring blood culture system prior to testing with the PhenoTest BC Kit.

Indeterminate, false positive and invalid results were obtained during the course of the LoD study. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

i. Interference Studies

The Accelerate PhenoTest BC kit ID and AST performance was compared using seeded positive blood culture samples with and without interfering substances. Organisms evaluated included one species from each of the major groups of organisms identified by the PhenoTest BC kit: *E. faecalis, S. aureus, S. agalactiae, S. pneumoniae, A. baumannii, E. coli, P. aeruginosa, S. marcescens* and *C. albicans*. Both endogenous substances and heparin were evaluated as possible interferents. Endogenous substances are listed in Table 24 below. The interference of RBCs was tested using 2 bottle types (BACTEC Plus Aerobic/F Medium and BACTEC Lytic/10 Anaerobic/F Medium). The remaining substances were tested using standard BACTEC Plus Aerobic/F Medium bottles. All interfering substances were co-spiked with the isolate and incubated until positivity in the continuous monitoring blood culture instrument. Each set included a control (contrived positive blood culture with no added endogenous substance or antibiotic) and replicates of the same contrived sample spiked with one of the interfering substances. This study was designed to allow for additional (stage 2) testing for any organism/antimicrobial/interferent combination for which discrepancies were observed.

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 Accelerate PhenoTest BC Kit.*

Table 24. Potential Interferents, Concentration Tested and Reference Range for Normal

Potential Interferent	Concentration Tested	Reference Range	
RBCs (Hematocrit/Hemoglobin)	20g/dL	Normal	1-2 g/L
WBCs (buffy coat)	12,000 WBC/µL	Normal adult	4,500 - 10,000/µL
		Severe sepsis	>12,000/µL or <4000/µL
Platelets	400,000/ µL	Normal adult	150,000 - 400,000/ µL
		Thrombocytopenia	<150,000
		Severe sepsis	80.000/µL
Triglycerides	37 mmol/L	0.34 – 3.7 mmol/L	
Bilirubin (conjugated)	34.2 µmol/L	0 – 3.4 µmol/L	
Gamma Globulin	50 mg/mL	6 – 13 g/L	
Heparin	3000	350 – 1000 U/L	

Identification Results. Results obtained for the identification assay showed 99.5% agreement with expected results and all samples returned an identification result.

AST Results. Endogenous substances/organism/antimicrobial combinations demonstrated greater than 89.9% EA for 98% (352 out of 360) of combinations and greater than 89.9% CA for 94% (340 out of 360) of combinations as compared to controls not containing potentially interfering substances.

The results provided for the identification assay and AST assay with endogenous substances and heparin are acceptable.

Indeterminate, false positive and invalid results were obtained during the course of the interfering substances study. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

j. *Blood Bottle Type*

Identification Assay

Thirteen different blood culture bottle types from three different blood culture systems (BacT/Alert , (bioMérieux, Inc.) BACTEC (Becton Dickinson) and VersaTREK (ThermoFisher) were evaluated analytically with the Accelerate Pheno Assay (See Table 25 below). Blood culture bottles/media were tested with the recommended ratio of blood to media. Replicates of the following organisms were inoculated into blood culture bottles: *A. baumannii, E. coli, P. aeruginosa, S. aureus, S. pneumoniae, E. faecalis* and *C. albicans*. In addition, an off-panel organism, *Micrococcus luteus,* was tested with all bottle types. Organisms were inoculated into the bottles at concentrations informed by results obtained in the Growth and Detection study and incubated on the appropriate automated blood culture instrument until positive detection. Positive bottles were removed from the blood culture instrument and samples were immediately run on the Accelerate PhenoTest BC Kit.

All samples were evaluated with a minimum of 10 replicates (Stage 1 testing); additional replicates were tested if the expected organism was not detected or if additional testing was required to provide a sufficient number of valid results for each antimicrobial agent (Stage 2 testing).

For the identification assay, results were considered acceptable if the organism inoculated into the bottle was detected and correctly identified. Tests that gave invalid results (too few cells in the probe channels) were not included in the evaluation. For 12 of 13 bottle types the identification assay provided acceptable results (>95% of organisms detected and correctly identified). One bottle type, BACTEC PLUS Anaerobic F detected and identified 94.3% of aerobic isolates due to non-detection of *C. albicans* in 3/6 replicates. The off-panel organism, *M. luteus* gave invalid results for the majority of tests; detection of off-panel organisms is not included in the claim for the PhenoTest BC Kit.

Results below represent cultures that were determined to be positive by the blood culture system and provided a valid result.

Table 25. Blood Bottle Study Results – Identification Assay

Blood Bottle Type	True Positives No. Detected/No. Valid Results (%) (All Organisms)	Species Not Detected No. Not Detected/No. Tested
BacT/ALERT		
SA Standard Aerobic	121/121 (100.0)	-
SN Standard Anaerobic	108/108 (100.0)	-
FA Plus Aerobic	110/111 (99.1)	*P. aeruginosa* (1/14)
FN Plus Anaerobic	112/116 (96.6)	*C. albicans* (3/13) *P. aeruginosa* (1/13)
PF Plus	125/128 (97.7)	*C. albicans* (3/30)

Blood Bottle Type	True Positives No. Detected/No. Valid Results (%) (All Organisms)	Species Not Detected No. Not Detected/No. Tested
BACTEC		
Standard/10 Aerobic F	115/118 (97.5)	*P. aeruginosa* (3/22)
Standard Anaerobic /F	58/58 (100.0)	-
Plus Aerobic F	165/172 (95.9)	*C. albicans* (1/26) *E. faecalis* (1/22) *P. aeruginosa* (5/45)
Plus Anaerobic/F	66/70 (94.3)	*C. albicans* (3/6) *S. aureus* (1/21)
PEDS PLUS/F	137/141 (97.2)	*C. albicans* (2/26) *E. faecalis* (2/21)
Lytic/10 Anaerobic/F	69/72 (95.8)	*E. faecalis* (3/23)
VersaTREK		
REDOX 1 (aerobic)	129/132 (97.7)	*E. faecalis* (1/21) *P. aeruginosa* (2/37)
REDOX 2 (anaerobic)	63/64 (98.4)	*A. baumannii* (1/2)

AST

For the AST assay results of each bottle type with each antimicrobial/organism combination were compared to the modal MIC value obtained with the broth microdilution reference method. In addition, results for each antimicrobial/organism combination were compared across all bottles types and compared to the modal MIC obtained with the Pheno System.

For results compared to the modal MIC of the BMD reference method, all bottle types provided acceptable EA and CA values for most organisms tested.

For detection of MLSb resistance a sufficient number of tests were performed only with the following bottle types: BacT/ALERT SA Standard Aerobic, BacT/Alert FA Plus Aerobic, BacT/ALERT FN Plus Anaerobic, BacT/ALERT PF Plus, BACTEC Plus Aerobic F and BACTEC Lytic/10 Anaerobic/F. An insufficient number of tests were performed with the remaining blood culture bottle types. The sponsor included the following limitation in their device labeling:

> *Due to an insufficient number of test isolates, the ability of the Accelerate PhenoTest BC kit to detect inducible MLSb resistance in coagulase-negative staphylococci is unknown when used with the following blood culture bottle types: BacT/Alert SN Standard Anaerobic, BACTEC Peds Plus/F, BACTEC Plus Anaerobic/F, BACTEC Standard Anaerobic, BACTEC Standard/10 Aerobic, VersaTrek Redox 1 Aerobic, VersaTrek Redox 2 Anaerobic. Use an alternative method for detection of inducible MLSb resistance when using these blood culture bottle types if critical to patient care.*

Indeterminate, false positive and invalid results were obtained during the course of the blood bottle study. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

k. *Polymicrobial LoD*

The purpose of the Polymicrobial Limit of Detection Study was to characterize the levels of different microorganisms that must be present in blood cultures containing two different microbial species in order for the PhenoTest BC Kit to perform antimicrobial susceptibility testing on either or both species. Testing was performed using 13 pairs of different microbial species. Individual strains were inoculated into blood culture bottles containing whole human blood and incubated until called positive by an automated blood culture instrument. The positive blood cultures were then mixed in different ratios to obtain simulated polymicrobial cultures at different target levels. The concentration of each organism in each mixture was determined by performing viable counts on the parental monomicrobial cultures. Each mixture was tested in duplicate using the PhenoTest assay.

Results of the identification assay showed acceptable detection and identification of all isolates in the various concentrations. Only *S. aureus* (present in concentrations equivalent to that at positivity) with high concentrations of *C. albicans* (three to four log higher concentration than S. aureus) was not detected.

AST testing of two organisms was not supported by the test results. The PhenoTest BC Kit will only perform AST testing on a single isolate in a polymicrobic sample. See above for details on AST testing in polymicrobic samples.

Indeterminate, false positive and invalid results were obtained during the course of the polymicrobial LoD study. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

l. *Biological interference*

The potential for biological interference of polymicrobial positive blood culture samples was evaluated by testing 17 combinations of various resistant isolates with previously established modal MICs or known resistance phenotypes in the presence of other isolates. Testing was performed at the time of positive detection by a continuous monitoring blood culture system. Testing was limited to polymicrobial samples containing 2 target species and included on-panel and off-panel species (*Propionibacterium acnes, Bacteroides fragilis* and *Stenotrophomonas maltophilia)*.

For the identification assay 15 of 17 combinations resulted in 100% detection of all organisms. The results of the study are shown in Table 26 below. Results of the identification assay showed acceptable detection and identification of all isolates in the various concentrations. Only *S. aureus* and *K. pneumoniae* (present in concentrations equivalent to that at positivity) with high concentrations of *C. albicans* (three to four log higher concentration than *S. aureus*) was not detected.

AST testing demonstrated biological interference; AST results will only be reported for one organism in polymicrobial samples. See Section I (Algorithm for Performance of AST) above for details on AST testing in polymicrobic samples.

Table 26. Organism Combinations Tested to Determine Biological Interference

Combination	Species 1	Number Identified/Total (% Identified)	Species 2	Number Identified/Total (%)
Combination 1	*S. aureus*	8/8 (100.0%)	*K. pneumoniae*	8/8 (100.0%)
Combination 2	*S. aureus*	2/2 (100.0%)	*K. pneumoniae*	2/2 (100.0%)
Combination 3	*S. aureus*	4/4 (100.0%)	*E. cloacae*	4/4 (100.0%)
Combination 4	*S. aureus*	3/3 (100.0%)	*K. pneumoniae*	3/3 (100.0%)
Combination 5	*S. aureus*	2/2 (100.0%)	*P. acnes*	Not Detected
Combination 6	*S. aureus*	1/2[a] (50.0%)	*C. albicans*	2/2 (100.0%)
Combination 7	*S. epidermidis*	2/2 (100.0%)	*K. pneumoniae*	2/2 (100.0%)
Combination 8	*S. pneumoniae*	3/3 (100.0%)	*E. coli*	3/3 (100.0%)
Combination 9	*E. faecalis*	2/2 (100.0%)	*E. coli*	2/2 (100.0%)
Combination 10	*E. faecalis*	2/2 (100.0%)	*A. baumannii*	2/2 (100.0%)
Combination 11	*E. faecium*	2/2 (100.0%)	*E. aerogenes*	2/2 (100.0%)
Combination 12	*E. faecium*	6/6 (100.0%)	*A. baumannii*	6/6 (100.0%)
Combination 13	*E. faecium*	2/2 (100.0%)	*P. acnes*	Off-Panel Not Detected
Combination 14	*E. coli*	2/2 (100.0%)	*B. fragilis*	Off-Panel Not Detected
Combination 15	*A. baumannii*	2/2 (100.0%)	*S. maltophilia*	Off-panel Not Detected
Combination 16	*K. pneumoniae*	1/3[a] (33.0%)	*C. albicans*	3/3 (100.0%)

[a] *Candida* spp. positive blood cultures contain low levels of organism relative to other on panel identification organisms. Significant dilution of species paired with *Candida* spp. is required to achieve 1:1 ratio of organisms.

Indeterminate, false positive and invalid results were obtained during the course of the biological interference study. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

2. <u>Comparison studies:</u>

   a. *Method comparison with predicate device*
   Not applicable

   b. *Matrix comparison:*
   Not Applicable

3. <u>Clinical studies</u>:

The purpose of the clinical study was to demonstrate the clinical performance of the Accelerate PhenoTest BC system for positive blood culture identification and AST compared to reference results in a clinical setting. The clinical performance was established in a multi-center study conducted at 13 geographically distinct U.S. sites over approximately six months. Samples included prospectively collected fresh samples (aliquots of left-over positive blood cultures from patients suspected of bacteremia or fungemia and tested within eight hours of positivity), fresh seeded samples (blood cultures seeded with human blood and previously characterized fresh

clinical isolates isolated within seven days of seeding and incubated until positivity) and seeded samples (blood cultures seeded with human blood and previously characterized challenge and stock isolates and incubated until positivity). Challenge isolates were selected to represent organism/antimicrobial combinations that were less common and underrepresented in the fresh and fresh seeded samples and to provide resistant or on-scale MIC results for at least one antimicrobial agent. Challenge isolates were obtained from culture collections; stock isolates were obtained from clinical specimens at the clinical sites and represented isolates that had been stored for longer than seven days.

Samples were tested in the following bottle types based on the type of continuously monitored blood culture system in use at the testing site: BacT/ALERT SA Standard Aerobic, BacT/ALERT SN Standard Anaerobic, BACTEC Lytic/10 Anaerobic/F Medium, BACTEC PEDS PLUS/F Medium, BACTEC PLUS Aerobic/F Medium, BACTEC PLUS Anaerobic Medium, BACTEC Standard Anaerobic/F Medium, BACTEC Standard Aerobic/F Medium, VersaTREK REDOX 1 Aerobic Media (80 mL), VersaTREK REDOX 2 Anaerobic Media (80 mL).

Testing was initiated within eight hours of bottle ring; bottles flagged as positive were held at room temperature until testing.

Results of the identification assay were compared to results obtained with VITEK2 (bioMérieux) using isolates subcultured from the blood culture bottles tested with the Accelerate PhenoTest BC Kit. For isolates for which an invalid result was obtained by VITEK2, the reference identification was determined by 16S rRNA gene sequencing.

Results of the AST assay were compared to the CLSI broth microdilution reference method. Isolates were tested in triplicate by the broth microdilution method; the reference result was the mode MIC value determined through replicate testing. If no mode value could be determined or if a QC failure occurred, an additional three replicates were tested to determine the mode MIC. Performance of the evaluation of resistance markers (cefoxitin susceptibility and MLSb) was compared to disk diffusion performed singly; if the test failed or if the zone diameter was within one millimeter of the breakpoint, testing was repeated in triplicate with the modal category used as the reference result. All reference testing was performed at a reference laboratory.

A total of 2500 positive blood cultures were enrolled in the study. Samples were excluded from the ID assay study for the following reasons: protocol deviations, samples that halted workflow, samples enrolled but not tested, samples run outside of the eight hour post-positivity window, samples with no organism identified by Gram stain or subculture or samples for which isolates were considered unacceptable upon receipt at the reference laboratory, samples for which the Accelerate Pheno system did not complete the assay or for which results were invalid. Samples were excluded from the AST assay for the following reasons: AST testing performed after completion of collection of adequate data for AST, samples with no broth microdilution reference data collected, samples for which PhenoTest BC kit did not collect AST data, samples with discordant identifications between PhenoTest BC kit and the reference method, samples with invalid results, samples for which two isolates with the same identification were obtained.

Results were evaluated for a total of 1850 positive blood culture samples including 793 fresh samples, 65 fresh seeded samples, 477 samples seeded with challenge isolates and 515 samples seeded with stock isolates.

The mean time from assay start to ID result was 1.38 hours; the mean time from assay start to AST result was 6.69 hours.

Results obtained from the PhenoTest BC kit should be interpreted in conjunction with Gram stain results obtained from the positive blood culture sample to mitigate the occurrence of false positive results. To address the need for concurrence of PhenoTest BC kit results with Gram Stain results the following limitations were included in the device labeling:

> *Due to the possibility of cross reactivity, all Accelerate PhenoTest BC kit results should be interpreted in conjunction with Gram stain*
>
> *Accelerate PhenoTest BC kit identification results that are discordant with the result of the blood culture Gram stain (for example, no organism detection when the Gram stain is positive or detection of a Gram-positive cocci when Gram-positive cocci were not observed in the Gram stain) should be confirmed by culture prior to reporting the test result. For some polymicrobic calls, false positive results may not be mitigated by Gram stain analysis (for example, detection of two Enterobacteriaceae species with Gram-negative rods observed in the Gram stain). Results of such polymicrobic calls should be verified by subculture and/or an alternative identification method.*

The use of the PhenoTest BC kit does not eliminate the need for subculture of the positive blood culture. The following limitation was included in the device labeling to address the need for subculture for certain PhenoTest BC kit results:

> *Subculture of positive blood culture is required in the following situations:*
>
> - *For the identification and susceptibility testing of off-panel organisms not identified by the Accelerate PhenoTest BC kit,*
> - *For samples that give a polymicrobial result*
> - *For organisms for which species identification is critical for patient care, (e.g. speciation of streptococci)*
> - *For testing antimicrobial agents not included on the Accelerate panel*
> - *For testing certain antimicrobial agents as discussed in AST limitations below*
> - *For testing samples for which an "indeterminate" result for any probe was obtained*
> - *To obtain isolates for epidemiologic testing.*

In addition, the PhenoTest BC kit does not provide species identification for members of *Streptococcus* (STR probe). Species detected by the STR probe include *S. mitis, S. oralis*, *S. gallolyticus, S. agalactiae* and *S. pneumoniae*. Subculture and alternative identification methods should be employed to determine the presence of *S. pneumoniae*. The following limitation was included in the device labeling:

> *Additional subculture is required for the identification of S. pneumoniae in cases of a positive Streptococcus spp. call.*

a. Clinical Sensitivity and Specificity, Identification Assay

For identification results, performance was evaluated by calculating sensitivity and specificity (for prospectively collected fresh samples) or PPA and NPA (for seeded samples). The

performance of the ID assay for each of the target probes is presented in Tables 27-29 below for the 1850 analyzed samples.

Table 27. Summary of Identification Assay Performance, Gram-Positive Organisms

Probe	Specimen Type	Sensitivity/PPA			Specificity/NPA		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
CNS	Prospective Fresh	145/157	92.4	(87.1-95.6)	595/609	97.7	(96.2-98.6)
	Seeded	98/98	100	(96.2-100.0)	863/876	98.5	(97.5-99.1)
	Total	243/255	95.3	(92.0-97.3)	1458/1485	98.2	(97.4-98.8)
EFS	Prospective Fresh	33/35	94.3	(81.4-98.4)	748/748	100.0	(99.5-100.0)
	Seeded	65/66	98.5	(91.9-99.9)	978/980	99.8	(99.3-100.0)
	Total	98/101	97.0	(91.6-99.0)	1726/1728	99.9	(99.6-100.0)
EFM	Prospective Fresh	20/20	100	(83.89-100.0)	762/770	99.0	(98.0-99.5)
	Seeded	80/82	97.6	(91.5-99.3)	962/969	99.3	(98.5-99.7)
	Total	100/102	98.0	(93.1-99.5)	1724/1739	99.1	(98.6-99.5)
SAU	Prospective fresh	173/178	97.2	(93.6-98.8)	586/597	98.2	(96.7-99.0)
	Seeded	65/65	100	(94.4-100.0)	962/975	98.7	(97.7-99.2)
	Total	238/243	97.9	(95.3-99.1)	1548/1572	98.5	(97.7-99.0)
SLU	Prospective fresh	1/2	50.0	(2.6-97.4)	784/784	100	(99.5-100.0)
	Seeded	76/77	98.7	(93.0-99.9)	964/965	99.9	(99.4-100.0)
	Total	77/79	97.5	(91.2-99.3)	1748/1749	99.9	(99.7-100.0)
STR	Prospective fresh	37/40	92.5	(80.1-97.4)	711/741	96.0	(94.3-97.2)
	Seeded	134/136	98.5	(94.8-99.6)	904/913	99.0	(98.1-99.5)
	Total	171/176	97.2	(93.5-98.8)	1615/1654	97.6	(96.8-98.3)

Table 28. Summary of Identification Assay Performance, Gram-Negative Organisms

Probe	Specimen Type	Sensitivity/PPA			Specificity/NPA		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
ABA	Prospective fresh	3/3	100	(43.9-100.0)	777/780	99.6	(98.9-99.9)
	Seeded	65/66	98.5	(91.9-99.9)	987/990	99.7	(99.1-100.0)
	Total	68/69	98.6	(92.2-99.9)	1764/1770	99.7	(99.3-99.8)
CIT	Prospective fresh	5/5	100	(56.6-100.0)	773/778	99.4	(98.5-99.7)
	Seeded	87/90	96.7	(90.7-98.9)	904/911	99.2	(98.4-99.6)
	Total	92/95	96.8	(91.1-98.9)	1677/1689	99.3	(98.8-99.6)
ENT	Prospective fresh	12/14	85.7	(60.1-96.0)	759/764	99.4	(98.5-99.7)
	Seeded	95/96	99.0	(94.3-100.0)	923/927	99.6	(98.9-99.8)
	Total	107/110	97.3	(92.29-99.07)	1682/1691	99.5	(98.99-99.72)
ECO	Prospective fresh	118/121	97.5	(93.0-99.2)	662/665	99.6	(98.7-99.9)
	Seeded	26/27	96.3	(81.7-99.8)	1028/1030	99.8	(99.3-100.0)
	Total	144/148	97.3	(93.3-98.9)	1690/1695	99.7	(99.3-99.9)
KLE	Prospective fresh	62/66	93.9	(85.4-97.6)	714/715	99.9	(99.2-100.0)
	Seeded	60/61	98.4	(91.3-99.9)	937/942	99.5	(98.8-99.8)
	Total	122/127	96.1	(91.1-98.3)	1651/1657	99.6	(99.2-99.8)
PRO	Prospective fresh	10/11	90.9	(62.3-99.5)	779/779	100.0	(99.5-100.0)
	Seeded	76/77	98.7	(93.0-99.9)	972/979	99.3	(98.5-99.7)

Probe	Specimen Type	Sensitivity/PPA			Specificity/NPA		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
	Total	86/88	97.7	(92.1-99.4)	1751/1758	99.6	(99.2-99.8)
PAE	Prospective fresh	19/19	100	(83.2-100.0)	766/771	99.4	(98.5-99.7)
	Seeded	39/39	100	(91.0-100.0)	1013/1018	99.5	(98.9-99.8)
	Total	58/58	100	(93.8-100.0)	1779/1789	99.4	(99.0-99.7)
SMA	Prospective fresh	10/10	100	(72.3-100.0)	782/782	100.0	(99.5-100.0)
	Seeded	41/41	100	(91.4-100.0)	1013/1015	99.8	(99.3-100.0)
	Total	51/51	100	(93.0-100.0)	1795/1797	99.9	(99.6-100.0)

Table 29. Summary of Identification Assay Performance, *Candida* spp.

Probe	Specimen Type	Sensitivity (PPA)			Specificity (NPA)		
		TP/(TP+ FN)	%	95% CI	TN/(TN+FP)	%	95% CI
CAL	Prospective fresh	4/4	100.0	(51.0-100.0)	777/780	99.6	(98.9-99.9)
	Seeded	41/41	100.0	(91.4-100.0)	1000/1005	99.5	(98.8-99.8)
	Total	45/45	100.0	(92.1-100.0)	1777/1785	99.6	(99.1-99.8)
CGL	Prospective fresh	10/10	100.0	(72.3-100.0)	752/768	97.9	(96.6-98.7)
	Seeded	40/40	100.0	(91.2-100.0)	1002/1015	98.7	(97.8-99.3)
	Total	50/50	100.0	(92.9-100.0)	1754/1783	98.4	(97.7-98.9)

Monomicrobic and Polymicrobic Calls

The ability of the PhenoTest BC kit to determine if a sample contains a single organism (monomicrobic) and the ability to detect polymicrobic samples was evaluated with prospectively collected fresh samples (Tables 30 and 31). For monomicrobial results the PhenoTest BC Kit uses multiple tests and an algorithm driven analysis to determine that a sample contains a single organism with no evidence of additional organisms. Result reports for samples determined to be "monomicrobic" contain the following comment: "*Monomicrobial: Sample positive for only one pathogen*". Of 793 fresh prospective samples evaluated with PhenoTest BC, 557 were determined to be monomicrobial by the PhenoTest BC kit. Of the samples with a PhenoTest BC monomicrobial call, 545 were also monomicrobial by the reference method giving a 97.8% agreement for the PhenoTest BC kit monomicrobial call as compared to the reference method. Twelve samples were incorrectly identified as monomicrobial by PhenoTest BC; after Gram stain mitigation (seven samples clearly mitigated by bacillus/cocci morphology, three samples with gram-positive cocci potentially mitigated by cell arrangement, cocci in chains vs. cocci in clusters) two samples remained unmitigated (Table 30).

Seven of 38 samples determined to be polymicrobic by the reference method were also found to be polymicrobic by the PhenoTest BC kit (Table 31). The reference method reported 755 monomicrobic prospectively collected samples (95.2% of all prospective samples). Of these, 664 were potentially monomicrobic by the PhenoTest BC kit (including monomicrobic, indeterminate and false positive calls.)

Table 30. Performance of Monomicrobial Calls, Fresh Prospective Samples

Total No. Prospective Fresh Samples	793
Total No. Monomicrobial by PhenoTest BC Kit (with no evidence of additional organisms detected)	557
No. Monomicrobial by PhenoTest BC Kit and Reference Method	545
Percent Correct by PhenoTest BC	97.8
No. False Monomicrobial by PhenoTest BC	12
No. Mitigated by Gram Stain reaction (gram-positive vs. gram-negative)	7
No mitigated by gram stain by cellular morphology	3
No. unmitigated by Gram stain	2

Table 31. Fresh Prospective Samples by Outcome

Outcome	Reference	PhenoTest BC Kit
No. Fresh Prospective Samples (%)	793	872
No. Monomicrobic	755 (95.2)	664 (76.1)[b]
No. Polymicrobic	38 (4.8)	7 (0.8)[b]
False Positive	N/A	95 (10.9)[b]
Indeterminate[a]	N/A	27 (3.1)[b]
Invalid	N/A	79 (9.1)[b]

[a] Samples with only indeterminate results. Samples with a combination of valid and indeterminate results are included in the monomicrobic, polymicrobic or false positive categories

[b] PhenoTest BC Kit prospective outcome rates are calculated considering valid and invalid results for a total of 872

False Positives, Invalid and Indeterminate Results

Indeterminate, false positive and invalid results were obtained during the course of the clinical study. See Tables 35 and 36 for summaries of the indeterminate results. See Tables 37 and 38 for a summary of the false positive and invalid results, respectively.

AST Results

For the AST assay, performance was determined generally based on criteria outlined in the Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems including essential agreement (EA) and categorical agreement (CA) with results determined by replicate testing of the broth microdilution reference method. The number and percent of major and very major errors was also evaluated. Essential agreement was calculated as the percentage of MIC results that fell within ± 1 doubling dilution of the broth microdilution result; CA was calculated as the percentage of interpretive categories that exactly matched the interpretive categories obtained with the broth microdilution method.

For MIC values that are on-scale, the percent EA of evaluable results was determined. This assessment was only performed for those drugs for which the number of evaluable results was at least 55% of the total number of results. Analysis was also performed to determine MIC trending (higher or lower dilution compared to the reference MIC values).

Ampicillin. A total of 238 *Enterococcus* spp. isolates were evaluated with ampicillin (96 *E. faecium* isolates, and 142 *E. faecalis* isolates). The combined results from clinical and challenge testing demonstrated an EA of 100% and CA of 99.6% (Table 32). There was a single major error with *E. faecium* that was considered a random error.

Ceftaroline. A total of 344 *S. aureus* isolates were evaluated with ceftaroline. The combined results from clinical and challenge testing demonstrated an EA of 93.3% and CA of 99.7% (Table 32). There were an insufficient number of resistant isolates tested which was addressed with the following limitation in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Ceftaroline -S. aureus*

Daptomycin. A total of 332 *Staphylococcus* isolates were evaluated with daptomycin (197 *S. aureus* isolates, 135 coagulase negative *Staphylococcus* isolates). There is only a susceptible breakpoint for daptomycin with *Staphylococcus* spp. (≤1 µg/mL). The combined results from clinical and challenge testing demonstrated an EA for *Staphylococcus* of 99.1% and CA of 99.7%. There was one very major error with a *S. aureus* isolate that was considered a random error; however, because an insufficient number resistant isolates of *S. aureus* isolates were evaluated during the clinical trial, the performance with resistant isolates is unknown. The following limitation was added to the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Daptomycin - S. aureus*

A total of 111 *Enterococcus* isolates were evaluated with daptomycin (71 *E. faecium* isolates, 40 *E. faecalis* isolates). There is only a susceptible breakpoint for daptomycin with *Enterococcus* spp. (≤4 µg/mL). The combined results from clinical and challenge testing demonstrated an EA for *Enterococcus* spp. of 95.5% and CA of 99.1% (Table 32). There was one major error with an *E. faecium* isolate which was considered a random error. Potential major errors with daptomycin are addressed with the following limitation in the device labeling:

> *The current absence of data on daptomycin-resistant isolates precludes defining any categories other than "Susceptible." Isolates yielding test results suggestive of a "Non-Susceptible" category should be retested, and if the result is confirmed, the isolate should be retested using the reference method.*

Erythromycin. A total of 194 S. aureus isolates were initially tested with erythromycin. While the EA and CA were acceptable, results showed a high occurrence of very major errors (5.9%). A correction was made to the AST model and regression analysis was used to validate results from the initial testing. Final analysis of erythromycin was made using a total of 338 *S. aureus* isolates were evaluated with erythromycin. The combined results from clinical and challenge testing demonstrated an EA of 98.2% and CA of 96.7% (Table 32). There was one major error that was considered a random error.

Linezolid. A total of 194 *S. aureus* isolates were evaluated with linezolid (breakpoints ≤4, - ≥8 µg/mL). The combined results from clinical and challenge testing demonstrated an EA for *S. aureus* of 99.5% and CA of 100% (Table 32). The EA of evaluable results for *S. aureus* was 99.5%. A total of 110 *Enterococcus* spp. isolates were evaluated (41 *E. faecalis* isolates, 69 *E. faecium* isolates) (breakpoints ≤2, 4, ≥8 µg/mL). The combined results from clinical and challenge testing demonstrated an EA for *Enterococcus* spp. of 96.4% and CA of 98.2% (Table 32). The EA of evaluable results for *Enterococcus* spp. was 100%.

Vancomycin. A total of 275 coagulase negative *Staphylococcus*, and *Enterococcus* spp. were evaluated with vancomycin (134 coagulase negative *Staphylococcus* isolates, 29 *S. lugdunensis* isolates, 41*E. faecalis* isolates and 71 *E. faecium* isolates) (breakpoints ≤4, 8-16, ≥32) µg/mL. The combined results from clinical and challenge testing demonstrated an EA for coagulase negative *Staphylococcus*, *S. lugdunensis* and *Enterococcus* spp. of 96.4% and CA of 96.4%. A total of 198 *S. aureus* isolates were evaluated with vancomycin (breakpoints ≤2, 4-8, ≥16 µg/mL). The combined results from clinical and challenge testing demonstrated an EA for *S. aureus* of 98.0% and CA of 99.0% (Table 32). There were an insufficient number of vancomycin-intermediate *S. aureus* (VISA) isolates evaluated and the following limitation was included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect vancomycin-intermediate Staphylococcus aureus isolates (VISA) is unknown because insufficient numbers of VISA isolates were evaluated at the time of comparative testing.*

Amikacin. A total of 432 isolates were evaluated with amikacin (47 *A. baumannii* isolates, 38 *Citrobacter* spp. isolates, 110 *E. coli* isolates, 51 *Enterobacter* spp. isolates, 78 *Klebsiella* spp. isolates, 31 *Proteus* spp. isolates, 35 *S. marcescens* isolates, 42 *P. aeruginosa* isolates). The combined results from clinical and challenge testing demonstrated an EA of 94.2% and CA of 94.0% (Table 33). There were an insufficient number of resistant isolates of *Citrobacter* sp., *Enterobacter* spp., *E. coli*, *Proteus* spp. and *S. marcescens* tested and the following limitation was included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Amikacin - Citrobacter spp., Enterobacter spp., E. coli, Proteus spp., S. marcescens*

Testing of *A. baumannii* with amikacin demonstrated an EA of 80.9% and CA of 80.9%. The low CA was due to a high number of minor errors. Isolates of A. baumannii that were out of EA with the reference method were isolates that gave resistant MIC values with PhenoTest BC but gave intermediate MIC results with the reference method. To address the low EA of *A. baumannii* with amikacin the following limitation was included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to provide accurate MICs with amikacin resistant strains of A. baumannii has not been established; isolates of this species that provide resistant results should be confirmed by an alternative method.*

Ampicillin/Sulbactam. A total of 322 isolates were evaluated with ampicillin/sulbactam (186 *E. coli* isolates, 93 *Klebsiella* spp. isolates, 43 *Proteus* spp. isolates). The combined results from clinical and challenge testing demonstrated an EA of 91.0% and CA of 84.2% (Table 33). The low CA was due to the occurrence of a high number of minor errors with *E. coli* and *Klebsiella* sp. A single very major error was observed with *Proteus* spp. and was considered a random error. For *Proteus* spp. the essential agreement was low at 81.4%. Analysis of performance of *Proteus* spp. using truncated reporting ranges improved the EA to 90.7%. Analysis of trending indicated that MIC values for *Enterobacteriaceae* tended to be one doubling dilution higher than the reference MIC value. The following statement was included as a footnote to the AST performance table:

Accelerate PhenoTest BC kit ampicillin/sulbactam MIC values for Enterobacteriaceae tended to be one doubling dilution higher than the reference MIC value.

Aztreonam. A total of 348 isolates of *Enterobacteriaceae* were evaluated with aztreonam (38 *Citrobacter* spp. isolates*, 124 E. coli* isolates, 39 *Enterobacter* spp. isolates, 73 *Klebsiella* spp. isolates, 38 *Proteus* spp. isolates, 36 *S. marcescens* isolates). The combined results from clinical and challenge testing demonstrated a combined EA of 96.6% and CA of 97.7% (Table 33). A single very major error with *E. coli* and a single major error with *Enterobacter* spp. were considered random errors. There were an insufficient number of resistant isolates of *Proteus* spp. *and S. marcescens* tested and the following limitation was included in the device labeling:

The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Aztreonam - Proteus spp., S. marcescens.

Analysis of trending indicated that MIC values for *Enterobacteriaceae* tended to be one doubling dilution higher than the reference MIC value. The following statement was added as a footnote to the AST performance table:

Accelerate PhenoTest BC kit aztreonam MIC values for Enterobacteriaceae tended to be one dilution higher than the reference MIC value.

Cefepime. A total of 349 isolates of *Enterobacteriaceae* were evaluated with cefepime (37 *Citrobacter* spp. isolates*, 124 E. coli* isolates, 40 *Enterobacter* spp. isolates, 74 *Klebsiella* spp. isolates, 37 *Proteus* spp. isolates, 37 *S. marcescens* isolates) (breakpoints ≤2, 4-8, ≥16 µg/mL). The combined results from clinical and challenge testing demonstrated a combined EA of 97.7% and CA of 96.8% (Table 33). There was a single very major error observed with *E. coli* which considered a random error. There were an insufficient number of resistant isolates of *Citrobacter* spp., *Proteus* spp. *and S. marcescens* tested and the following limitation is included in the device labeling:

The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Cefepime - Citrobacter spp., Proteus spp., S. marcescens.

A total of 42 isolates of *P. aeruginosa* were evaluated with cefepime (breakpoints ≤8, -, ≥16 µg/mL). The combined results from clinical and challenge testing demonstrated a combined EA of 88.1% and CA of 92.9% which was considered to be acceptable performance (Table 33). The EA of evaluable results for *P. aeruginosa* was 83.3%. Three major errors observed with *P. aeruginosa* and were addressed with the following limitation in the device labeling:

Cefepime with P. aeruginosa may produce a resistant result that can be found susceptible by the reference method. If critical to patient care confirm these results with an alternate method.

Ceftazidime. A total of 377 isolates of *Enterobacteriaceae* were evaluated with ceftazidime (74 *Citrobacter* spp. isolates*, 122 E. coli* isolates, 40 *Enterobacter* spp. isolates, 69 *Klebsiella* spp. isolates, 38 *Proteus* spp. isolates, 34 *S. marcescens* isolates) (breakpoints ≤4, 8, ≥16 µg/mL). The combined results from clinical and challenge testing demonstrated an EA of 86.2% and a CA of

93.9% (Table 33). Analysis of performance using truncated reporting ranges improved the EA for *Enterobacteriaceae* to 93.1%. Essential agreement of evaluable results was 77.4% for *Enterobacteriaceae*. There were an insufficient number of resistant isolates of *Proteus* spp. *and S. marcescens* tested and the following limitation was included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Ceftazidime-Proteus spp., S. marcescens.*

Analysis of trending indicated that MIC values for *Enterobacteriaceae* tended to be one doubling dilution higher than the reference MIC value. The following statement was added as a footnote to the AST performance table:

> *Accelerate PhenoTest BC kit ceftazidime MIC values for Enterobacteriaceae tended to be one doubling dilution higher than the reference method.*

A total of 53 isolates of *P. aeruginosa* were evaluated with ceftazidime (breakpoints ≤8, -, ≥16 µg/mL). The combined results from clinical and challenge testing demonstrated an EA of 86.8% and CA of 88.7% (Table 33). Analysis of performance using truncated reporting ranges improved the EA to 90.6%. There were six major errors observed with *P. aeruginosa* and were addressed with the following limitation in the device labeling:

> *Ceftazidime with P. aeruginosa may produce a resistant result that can be found susceptible by the reference method. If critical to patient care confirm these results with an alternate method. Any P. aeruginosa isolates that provides an MIC ≥ 16µg/mL should be retested with an alternate method.*

Analysis of trending indicated that MIC values for *P. aeruginosa* tended to be one doubling dilution lower than the reference MIC value. The following statement was added as a footnote to the AST performance table:

> *Accelerate PhenoTest BC kit ceftazidime MIC values for P. aeruginosa tended to be one doubling dilution lower than the reference method.*

Ceftriaxone. A total of 324 isolates of *Enterobacteriaceae* were evaluated with ceftriaxone (30 *Citrobacter* spp. isolates*, 111 E. coli* isolates, 40 *Enterobacter* spp. isolates, 70 *Klebsiella* spp. isolates, 33 *Proteus* spp. isolates, 40 *S. marcescens* isolates). The combined results from clinical and challenge testing demonstrated an EA of 89.8% and a CA of 96.6% which was determined to be acceptable (Table 33). For *Proteus* spp. with ceftriaxone the essential agreement was low at 87.7%. Analysis of performance using truncated reporting ranges improved the EA for *Proteus* spp. to 93.9%. There were an insufficient number of resistant isolates of *Citrobacter* spp., *Enterobacter cloacae* and *S. marcescens* tested and the following limitation was included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Ceftriaxone - Citrobacter spp., E. cloacae, S. marcescens.*

The essential agreement of ceftriaxone with *S. marcescens* was unacceptable at 45.0%; CA was 82.5% due to a high number of minor errors. Truncation of the reporting range did not improve the %EA. The low EA for *S. marcescens* was addressed with the following limitation in the device labeling:

> *Due to a low essential agreement for S. marcescens with ceftriaxone, results should be confirmed with an alternate method if critical to patient care.*

Ciprofloxacin. A total of 394 isolates of *Enterobacteriaceae* and *P. aeruginosa* were evaluated with ciprofloxacin (38 *Citrobacter* spp. isolates*, 125 E. coli* isolates, 40 *Enterobacter* spp. isolates, 74 *Klebsiella* spp. isolates, 38 *Proteus* spp. isolates, 37 *S. marcescens* isolates, 42 *P. aeruginosa* isolates). The combined results from clinical and challenge testing demonstrated an EA of 96.7% and a CA of 98.2% (Table 33). For *Enterobacter* spp. with ciprofloxacin the EA was 85.0%; analysis of performance using truncated reporting ranges improved the EA for *Enterobacter* spp. to 100%. There were an insufficient number of resistant isolates of Citrobacter spp., *Proteus* spp. and *S. marcescens* tested and the following limitation was included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Ciprofloxacin - Citrobacter spp., Proteus spp., S. marcescens.*

Ertapenem. A total of 351 isolates of *Enterobacteriaceae* were evaluated with ertapenem (40 *Citrobacter* spp. isolates*, 125 E. coli* isolates, 40 *Enterobacter* spp. isolates, 71 *Klebsiella* spp. isolates, 38 *Proteus* spp., 37 *S. marcescens* isolates). The combined results from clinical and challenge testing demonstrated an EA of 98.9% and a CA of 98.6% (Table 33). There were two major errors with *Enterobacter* spp. which were addressed by the following limitation in the device labeling:

> *Ertapenem with Enterobacter spp. may produce a resistant result that can be found susceptible by the reference method. If critical to patient care, confirm these results with an alternate method.*

There were an insufficient number of resistant isolates of Citrobacter spp., *Proteus* spp. and *S. marcescens* tested and the following limitation was included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Ertapenem - Citrobacter spp., Proteus spp., S. marcescens.*

Analysis of trending indicated that MIC values for *Enterobacteriaceae* tended to be one doubling dilution higher than the reference MIC value. The following statement was added as a footnote to the AST performance table:

> *Accelerate PhenoTest BC kit ertapenem MIC values for Enterobacteriaceae tended to be one doubling dilution higher than the reference MIC value.*

Gentamicin. A total of 385 isolates of *Enterobacteriaceae* and *P. aeruginosa* were evaluated with gentamicin (39 *Citrobacter* spp. isolates, 122 *E. coli* isolates, 40 *Enterobacter* spp. isolates, 71 *Klebsiella* spp. isolates, 36 *Proteus* spp. isolates, 35 *S. marcescens* isolates, 42 P. aeruginosa isolates). The combined results from clinical and challenge testing demonstrated an EA of 99.2% and a CA of 98.4% (Table 33). The CA for *P. aeruginosa* was low at 88.1% due to a high number of minor errors. There were an insufficient number of resistant isolates of *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp. and *S. marcescens* tested and the following limitation is included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following organisms is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Citrobacter spp., Enterobacter spp., Proteus spp., S. marcescens.*

Meropenem. A total of 364 isolates of *Enterobacteriaceae* were evaluated with meropenem (39 *Citrobacter* spp. isolates, *124 E. coli* isolates, 57 *Enterobacter* spp. isolates, 71 *Klebsiella* spp. isolates, 37 *Proteus* spp. isolates, 36 *S. marcescens* isolates) (breakpoints ≤1, 2, ≥4 µg/mL). The combined results from clinical and challenge testing with *Enterobacteriaceae* demonstrated an EA of 97.0% and a CA of 98.1% (Table 33). For *Enterobacter* spp. the EA was low at 87.7%. Analysis of performance using truncated reporting ranges improved the EA for *Enterobacter spp.* to 91.2%. There was a single major error with *E. coli* that was considered to be a random error. There were three major errors with *Enterobacter* spp. which were addressed by the following limitation in the device labeling:

> *Meropenem with Enterobacter spp. may produce a resistant result that can be found susceptible by the reference method. If critical to patient care, confirm these results with an alternate method.*

There were an insufficient number of resistant isolates of *Citrobacter* spp., *E. coli*, *Proteus* spp. and *S. marcescens* tested and the following limitation is included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Citrobacter spp., E. coli, Proteus spp., S. marcescens*.

A total of 51 isolates of *P. aeruginosa* were evaluated with meropenem. The combined results from clinical and challenge testing with *P. aeruginosa* demonstrated an EA of 88.2 and a CA of 90.2% (Table 33) Analysis of performance using truncated reporting ranges improved the EA for *P. aeruginosa* to 90.2%. There was a single major error that was considered to be a random error.

Piperacillin/Tazobactam. A total of 519 isolates of *Enterobacteriaceae, A. baumannii* and *P. aeruginosa* were evaluated with piperacillin/tazobactam (73 *Citrobacter* spp. isolates*, 121 E. coli* isolates, 69 *Enterobacter* spp. isolates, 67 *Klebsiella* spp. isolates, 36 *Proteus* spp. isolates, 36 *S. marcescens* isolates, 47 *A. baumannii* isolates, 70 *P. aeruginosa* isolates). The combined results from clinical and challenge testing demonstrated an EA of 92.1% and a CA of 92.1% (Table 32). There was a single very major error with *E. coli* that was considered a random error. For *P. aeruginosa* the essential agreement was low at 85.7%. Analysis of performance using truncated reporting ranges improved the EA for *P. aeruginosa* to 91.4%. There was one major error with *A. baumannii* and two major errors with *Klebsiella* spp. The occurrence of major errors was

addressed in the following limitation in the device labeling:

> *Piperacillin/Tazobactam with Acinetobacter baumannii and Klebsiella spp. may produce a resistant result that can be found susceptible by the reference method. If critical to patient care, confirm these results with an alternate method.*

There were an insufficient number of resistant isolates of *Proteus* spp. and *S. marcescens* tested and the following limitation is included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following combination is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Piperacillin /Tazobactam - Proteus spp., S. marcescens.*

Tobramycin. A total of 389 isolates of *Enterobacteriaceae* and *P. aeruginosa* were evaluated with tobramycin (36 *Citrobacter* spp. isolates*, 122 E. coli* isolates, 40 *Enterobacter* spp. isolates, 74 *Klebsiella* spp. isolates, 38 *Proteus* spp. isolates, 37 *S. marcescens* isolates, 42 *P. aeruginosa* isolates). The combined results from clinical and challenge testing demonstrated an EA of 96.4% and a CA of 96.1% (Table 33). There were an insufficient number of resistant isolates of *Citrobacter* spp., *Proteus* spp. and *S. marcescens* tested and the following limitation is included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to detect resistance in the following organisms is unknown because an insufficient number of resistant isolates were encountered at the time of comparative testing: Citrobacter spp., Proteus spp., S. marcescens.*

Cefoxitin. A total of 398 *Staphylococcus* species were evaluated with cefoxitin to determine methicillin resistance (184 *S. aureus* isolates, 28 *S. lugdunensis* isolates, 180 coagulase negative *Staphylococcus* isolates). The combined results from clinical and challenge testing demonstrated CA of 98.2% (Table 34). Evaluation of fresh isolates of coagulase negative *Staphylococcus* spp. showed five very major errors; additional testing with 71 additional isolates of coagulase negative *Staphylococcus* demonstrated 100% CA with the reference method. Discordant analysis indicated that one isolate was falsely resistant by the reference method. Considering the lack of very major errors observed with repeat testing the performance of cefoxitin with coagulase negative *Staphylococcus* spp. was considered acceptable.

Macrolide-Lincosamide Streptogramim B Resistance (MLSb). A total of 164 *Staphylococcus* species were evaluated with MLSb to determine inducible clindamycin resistance (29 *S. lugdunensis* isolates, 135 coagulase negative *Staphylococcus* isolates). The combined results from clinical and challenge testing demonstrated CA of 98.2% (Table 34). The ability of the PhenoTest BC kit to determine inducible clindamycin resistance in *S. aureus* has not been established; the following limitation is included in the device labeling:

> *The ability of the Accelerate PhenoTest BC kit to provide accurate results for S. aureus with MLSb as compared to the reference method has not been established; isolates of this species should be tested by an alternative method.*

The ability of the PhenoTest BC kit to detect MLSb has not been established with all blood culture bottle types. The following limitation is included in the device labeling:

Due to insufficient number of test isolates, the ability of the Accelerate PhenoTest BC kit to detect inducible MLSb resistance in coagulase-negative staphylococci is unknown when used with the following blood culture bottle types: BacT/Alert SN Standard Anaerobic, BACTEC Peds Plus/F, BACTEC Plus Anaerobic/F, BACTEC Standard Anaerobic, BACTEC Standard/10 Aerobic, VersaTrek Redox 1 Aerobic, VersaTrek Redox 2 Anaerobic. Use an alternative method for detection of inducible MLSb resistance when using these blood culture bottle types if critical to patient care.

Table 32. AST Assay Performance, Gram-Positive Organisms

Sample Type	Tot	No. EA	EA %	No. CA	CA %	No. R or NS	min	maj	vmj
Ampicillin - *Enterococcus* spp.									
Fresh	46	46	100.0	46	100.0	15	0	0	0
Fresh Seeded	3	3	100.0	3	100.0	2	0	0	0
Challenge	189	189	100.0	188	99.5	75	0	1	0
Total	238	238	100.0	237	99.6	92	0	1	0
Ceftaroline – *S. aureus*									
Fresh	140	132	94.3	140	100.0	0	0	0	0
Fresh Seeded	20	18	91.0	20	100.0	0	0	0	0
Challenge	184	171	92.9	183	99.5	0	1	0	0
Total	344	321	93.3	343	99.7	0	1	0	0
Daptomycin – *Staphylococcus* spp.									
Fresh	258	255	98.8	257	99.6	1	0	0	1
Fresh Seeded	25	25	100.0	25	100.0	0	0	0	0
Challenge	49	49	100.0	49	100.0	0	0	0	0
Total	332	329	99.1	331	99.7	1	0	0	1
Daptomycin – *Enterococcus* spp.									
Fresh	48	47	97.9	48	100.0	0	0	0	0
Fresh Seeded	3	3	100.0	3	100.0	0	0	0	0
Challenge	60	56	93.3	59	98.3	0	0	1	0
Total	111	106	95.5	110	99.1	0	0	1	0
Erythromycin – *S. aureus*									
Fresh	140	139	99.3	135	96.4	76	5	0	0
Fresh Seeded	18	18	100.0	18	100.0	10	0	0	0
Challenge	180	175	97.2	174	96.7	119	4	1	0
Total	338	332	98.2	327	96.7	205	10	1	0
Linezolid *S. aureus*									
Fresh	139	138	99.3	139	100.0	0	0	0	0
Fresh Seeded	8	8	100.0	8	100.0	0	0	0	0
Challenge	47	47	100.0	47	100.0	0	0	0	0
Total	194	193	99.5	194	100.0	0	0	0	0
Linezolid – *Enterococcus* spp.									
Fresh	47	46	97.9	46	97.9	0	1	0	0
Fresh Seeded	3	3	100.0	3	100.0	0	0	0	0
Challenge	60	57	95.0	59	98.3	1	1	0	0
Total	110	106	96.4	108	98.2	1	2	0	0
Vancomycin – *S. aureus*									
Fresh	140	139	99.3	140	100.0	0	0	0	0
Fresh Seeded	8	7	87.5	8	100.0	0	0	0	0

Sample Type	Tot	No. EA	EA %	No. CA	CA %	No. R or NS	min	maj	vmj
Challenge	50	48	96.0	48	96.0	0	2	0	0
Total	198	194	98.0	196	99.0	0	2	0	0
Vancomycin – *S. lugdunensis*, Coagulase Negative *Staphylococcus*, *Enterococcus* sp.									
Fresh	160	158	98.8	158	98.8	14	2	0	0
Fresh Seeded	19	19	100.0	19	100.0	2	0	0	0
Challenge	88	80	90.9	80	90.9	44	8	0	0
Total	275	265	96.4	265	96.4	60	10	0	0

EA – Essential Agreement (+/- 1 dilution)
CA – Category Agreement
R or NS – Resistant or non-susceptible isolates
min – minor discrepancies
maj – major discrepancies
vmj – very major discrepancies

Essential Agreement (EA) occurs when there is agreement between the result of the reference method and that of the PhenoTest BC Kit within plus or minus one serial two-fold dilution of the antibiotic. Category Agreement (CA) occurs when the interpretation of the result of the reference method agrees exactly with the interpretation of the PhenoTest BC Kit.

Table 33. AST Assay Performance, Gram-Negative Organisms

Sample Type	Tot	No. EA	EA %	No. CA	CA %	No. R or NS	min	maj	vmj
Amikacin – *Enterobacteriaceae, A. baumannii, P. aeruginosa*									
Fresh	182	180	98.9	180	98.9	2	2	0	0
Fresh Seeded	20	20	100.0	20	100.0	0	0	0	0
Challenge	230	207	90.0	206	89.6	47	24	0	0
Total	432	407	94.2	406	94.0	49	26	0	0
Ampicillin/Sulbactam - *Enterobacteriaceae*									
Fresh	155	136	87.7	119	76.8	49	34	1	1
Fresh Seeded	16	14	87.5	14	87.5	8	2	0	0
Challenge	151	143	94.7	138	91.4	64	13	0	0
Total	322	293	91.0	271	84.2	121	49	1	1
Aztreonam – *Enterobacteriaceae*									
Fresh	179	173	96.6	175	97.8	26	2	1	1
Fresh Seeded	19	18	94.7	17	89.5	1	2	0	0
Challenge	150	145	96.7	148	97.8	61	2	0	0
Total	348	336	96.6	340	97.7	88	6	1	1
Cefepime – *Enterobacteriaceae*									
Fresh	180	175	97.2	174	96.7	21	5	0	1
Fresh Seeded	19	19	100.0	18	94.7	2	1	0	0
Challenge	150	147	98.0	146	97.3	40	4	0	0
Total	349	341	97.7	338	96.8	63	10	0	1
Cefepime – *P. aeruginosa*									
Fresh	12	10	83.3	10	83.3	2	0	2	0
Fresh Seeded	1	1	100.0	1	100.0	0	0	0	0
Challenge	29	26	89.7	28	96.6	17	0	1	0
Total	42	37	88.1	39	92.9	19	0	3	0
Ceftazidime – *Enterobacteriaceae*									
Fresh	175	147	84.0	159	90.9	25	16	0	0
Fresh Seeded	15	12	80.0	14	93.3	2	1	0	0
Challenge	187	166	88.8	181	96.8	81	6	0	0

Sample Type	Tot	No. EA	EA %	No. CA	CA %	No. R or NS	min	maj	vmj
Total	377	325	86.2	354	93.9	108	23	0	0
Ceftazidime – *P. aeruginosa*									
Fresh	12	7	58.3	6	50.0	1	0	6	0
Fresh Seeded	1	1	100.0	1	100.0	0	0	0	0
Challenge	40	38	95.0	40	100.0	27	0	0	0
Total	53	46	86.8	47	88.7	28	0	6	0
Ceftriaxone – *Enterobacteriaceae*									
Fresh	166	150	90.4	162	97.6	32	4	0	0
Fresh Seeded	14	14	100.0	14	100.0	2	0	0	0
Challenge	144	127	88.2	137	95.1	33	7	0	0
Total	324	291	89.8	313	96.6	107	11	0	0
Ciprofloxacin – *Enterobacteriaceae, P. aeruginosa*									
Fresh	193	186	96.4	187	96.9	48	6	0	0
Fresh Seeded	20	20	100.0	20	100.0	3	0	0	0
Challenge	181	175	96.7	180	99.4	50	1	0	0
Total	394	381	96.7	387	98.2	101	7	0	0
Ertapenem – *Enterobacteriaceae*									
Fresh	181	180	99.4	181	100.0	1	0	0	0
Fresh Seeded	19	19	100.0	19	100.0	0	0	0	0
Challenge	151	148	98.0	146	96.7	28	3	2	0
Total	351	347	98.9	346	98.6	29	3	2	0
Gentamycin – *Enterobacteriaceae* and *P. aeruginosa*									
Fresh	189	189	100.0	189	100.0	19	0	0	0
Fresh Seeded	18	18	100.0	18	100.0	4	0	0	0
Challenge	178	175	98.3	172	96.6	32	5	1	0
Total	385	382	99.2	379	98.4	55	5	1	0
Meropenem – *Enterobacteriaceae*									
Fresh	180	177	98.3	179	99.4	1	0	1	0
Fresh Seeded	19	19	100.0	19	100.0	0	0	0	0
Challenge	165	157	95.2	159	96.4	34	3	3	0
Total	364	353	97.0	357	98.1	35	3	4	0
Meropenem – *P. aeruginosa*									
Fresh	12	8	66.7	9	95.0	3	3	0	0
Fresh Seeded	1	1	100.0	1	100.0	0	0	0	0
Challenge	38	36	94.7	36	94.7	22	1	1	0
Total	51	45	88.2	46	90.2	25	4	1	0
Piperacillin/Tazobactam – *Enterobacteriaceae, P. aeruginosa, A. baumannii*									
Fresh	189	168	88.9	170	89.9	12	16	3	0
Fresh Seeded	18	17	94.4	17	97.4	0	0	1	0
Challenge	293	312	93.9	291	93.3	141	19	1	1
Total	519	478	92.1	478	92.1	153	35	5	1
Tobramycin – *Enterobacteriaceae, P. aeruginosa*									
Fresh	191	184	96.3	185	96.9	16	6	0	0
Fresh Seeded	19	16	84.2	17	89.5	2	2	0	0
Challenge	179	175	97.8	172	96.1	45	7	0	0
Total	389	375	96.4	374	96.1	63	15	0	0

Table 34. AST Assay Performance, Phenotypic Resistance

Sample Type	Total Tested	CA	%CA	Negative (S)	Positive (R)	maj	vmj
Cefoxitin – *S. aureus, S. lugdunensis*, Coagulase Negative *Staphylococcus* spp.							
Fresh	**228**	**222**	**97.4**	**109**	**119**	**1**	**5**
Seeded	**23**	**23**	**100.0**	**6**	**17**	**0**	**0**
Challenge	**141**	**140**	**99.3**	**32**	**109**	**1**	**0**
Total	**398**	**391**	**98.2**	**152**	**246**	**2**	**5**
MLSb – *S. lugdunensis*, Coagulase Negative *Staphylococcus* spp.							
Fresh	**119**	**117**	**98.3**	**62**	**57**	**1**	**1**
Seeded	**17**	**16**	**94.1**	**5**	**12**	**1**	**0**
Challenge	**28**	**28**	**100**	**27**	**1**	**0**	**0**
Total	**164**	**161**	**98.2**	**94**	**70**	**2**	**1**

Indeterminate Results

Indeterminate results are PhenoTest BC Kit results that indicate that the target group may or may not be present. Tests resulting in an indeterminate call show characteristics that are significantly different from a negative result but do not meet positive result requirements. Indeterminate results were obtained for all target probes but most commonly with the following probes: CNS, ENT, KLE, SAU and SLU. Table 35 presents the number and percent of indeterminate results obtained in the clinical study by target probe.

Table 35. Clinical Study Indeterminate Results by Probe

Probe	No. Indeterminate	IndeterminatePercent of Total Tests[a]
ABA	0	0.0%
CAL	6	0.3%
CGL	0	0.0%
CIT	0	0.0%
CNS	110	5.9%
ECO	0	0.0%
EFM	6	0.3%
EFS	4	0.2%
ENT	47	2.5%
KLE	55	3.0%
PAE	0	0.0%
PRO	0	0.0%
SAU	35	1.9%
SLU	22	1.2%
SMA	0	0.0%
STR	9	0.5%

[a] Total number of tests per probe = 1852

Table 36. Indeterminate Results by Study Type

Clinical/Analytical Study	No. Total Tests	No. Indeterminate (%)
Clinical Performance Evaluation Study	1850	272 (14.7%)
Reproducibility Study	1644	264 (16.1%)
Blood Bottle Type Study	1419	74 (5.2%)
Polymicrobial Biological Interference Study	49	10 (20.4%)
Growth and Detection Study	175	20 (11.4%)
Inclusivity and Exclusivity Study	253	37 (14.6%)
Interfering Substances Study	520	41 (7.9%)
Monomicrobial Limit of Detection	849	99 (11.7%)
Polymicrobial Limit of Detection	182	28 (15.4%)

False Positive Results

False positive results were observed in the clinical study and in all analytical studies. Most false positive results were the result of cross reactivity, including intra-genus cross reactivity with a specific probe. Because all PhenoTest BC Kit results are correlated with results of Gram stain of the positive blood culture, many false positive results are mitigated. The number and percent of false positives and the results of Gram stain mitigation are shown in Table 37 below.

Table 37. False Positive Results in Clinical and Analytical Studies

Clinical/Analytical Studies - False Positive Results				
Clinical/Analytical Study	**No. Total Tests**	**No. False Positives (%)**	**No. Mitigated False Positives (%)[a]**	**No. Unmitigated False Positives (%)**
Clinical Performance Evaluation Study – all specimen types	1850	179 (9.7)	106 (59.2)	73 (40.8)
Clinical – Challenge	477	38 (7.9)	29 (76.3)	9 (23.7)
Clinical – Fresh	793	95 (12.0)	51 (53.7)	44 (46.3)
Clinical – Fresh Seeded	65	4 (6.2)	1 (25.0)	3 (75.0)
Clinical - Stock	515	42 (8.2)	25 (59.5)	17 (40.5)
Reproducibility Study	1644	131 (8.0)	110 (84.0)	21 (16.0)
Blood Bottle Type Study	1077	64 (5.9)	56 (87.5)	8 (12.5)
Polymicrobial Biological Interference Study	49	2 (4.1)	1 (50.0)	1 (50.0)
Growth and Detection Study	144	5 (3.5)	3 (60.0)	2 (40.0)
Inclusivity and Exclusivity Study	318	81 (25.5)	24 (29.6)	57 (70.4)
Interfering Substances Study	526	41 (7.8)	33 (80.5)	8 (19.5)
Monomicrobial Limit of Detection	849	95 (11.2)	88 (92.6)	7 (7.4)
Polymicrobial Limit of Detection	182	6 (3.3)	3 (50.0)	3 (50.0)

[a] Gram stain mitigation includes differentiating enteric-like gram-negative rods and gram-negative cocco-bacilli suggestive of *Acinetobacter* spp. *Acinetobacter* sp. may not consistently demonstrate the gram-negative cocco-bacilliary morphology.

Invalid Tests.
Invalid results occur when too few cells are present in the control channel for the identification assay. No ID or AST results are reported and the Patient Report states: *"Too few cells for analysis. Perform alternate testing method for identification and susceptibility results."* In the analytical studies, non-standard sample preparation designed to control for organism concentration was employed which contributed to some invalid rates. Further, the distribution of organisms in these studies was different from what would be observed in clinical samples. Some invalid results were due to off-panel species. The occurrence of invalid tests is summarized in Table 38 below.

Table 38. Invalid Tests in Clinical and Analytical Studies

Study	Total No. Tests	No. Invalid (%)
Clinical Performance Evaluation Study	1940	90[a] (4.6)
Reproducibility	1644	11 (0.7)
Blood Bottle Type Stage 1	1159	80[b] (6.9)
Blood Bottle Type Stage 2	341	1 (0.3)
Polymicrobial Biological Interference	49	0 (0)
Growth and Detection	144	14[c] (9.7)
Inclusivity and Exclusivity	318	65[d] (20.4)
Interfering Substances	526	6[e] (1.1)
Monomicrobial Limit of Detection	849	69[f] (8.0)
Polymicrobial Limit of Detection	182	0 (0)

[a] 52 samples invalid due to off panel organisms
[b] 68 samples invalid due to off-panel organisms
[c] 6 samples invalid due to off-panel organisms
[d] 53 samples invalid due to off-panel organisms
[e] 6 samples invalid due to off-panel organisms
[f] 64 samples invalid due to off-panel organisms

c. *Other clinical supportive data (when a. and b. are not applicable):*
N/A

4. Clinical cut-off:
N/A

5. Expected values/Reference range:

Table 39. FDA Breakpoints for Each Antimicrobial Agent Included in the PhenoTest BC Kit

Antimicrobial	Organisms Tested	FDA Breakpoints		
		S	I	R
Amikacin	*Enterobacteriaceae, P. aeruginosa and A. baumannii*	≤ 16	32	≥ 64
Ampicillin	*Enterococcus spp.*	≤ 8	-	≥ 16
Ceftaroline	*S. aureus*	≤ 1	2	≥ 4
Ampicillin/Sulbactam	*Enterobacteriaceae*	≤ 8	16	≥ 32
Aztreonam	*Enterobacteriaceae*	≤ 4	8	≥ 16
Cefepime	*Enterobacteriaceae*	≤ 2	4 - 8	≥ 16
	P. aeruginosa	≤ 8	-	≥ 16

Antimicrobial	Organisms Tested	FDA Breakpoints		
		S	I	R
Ceftazidime	*Enterobacteriaceae*	≤ 4	8	≥ 16
	P. aeruginosa	≤ 8	-	≥ 16
Ceftriaxone	*Enterobacteriaceae*	≤ 1	2	≥ 4
Ciprofloxacin	*Enterobacteriaceae and P. aeruginosa*	≤ 1	2	≥ 4
Daptomycin	*Staphylococcus spp.*	≤ 1	-	≥ 2
	Enterococcus spp.	≤ 4	-	≥ 8
Ertapenem	*Enterobacteriaceae*	≤0.5	1	≥ 2
Erythromycin	*S. aureus*	≤0.5	1 - 4	≥ 8
Gentamycin	*Enterobacteriaceae and P. aeruginosa*	≤ 4	8	≥ 16
Linezolid	*S. aureus*	≤ 4	-	≥ 8
	Enterococcus spp.	≤ 2	4	≥ 8
Meropenem	*Enterobacteriaceae*	≤ 1	2	≥ 4
	P. aeruginosa	≤ 2	4	≥ 8
Piperacillin/Tazobactam	*Enterobacteriaceae*	≤ 16	32 - 64	≥ 128
Tobramycin	*Enterobacteriaceae and P. aeruginosa*	≤ 4	8	≥ 16
Vancomycin	*S. aureus*	≤ 2	4 - 8	≥ 16
	S. lugdunensis and Coagulase Negative *Staphylococcus*	≤ 4	8-16	≥ 32

M. Instrument Name:

Accelerate Pheno System

N. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ___X_____ or No ________

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes ________ or No ___X_____

   Instrument System
   The Accelerate Pheno system is comprised of the Accelerate Pheno instrument, software, host computer, analysis computer, and the Accelerate PhenoTest BC kit. This fully-integrated *in vitro* diagnostic system utilizes the following components for test samples:

   - One to four modules (where each module can run one patient sample independently)
   - Automated pipetting system for transfer of sample and reagents to appropriate cassette flowcell channels in a predetermined sequence

- Incubation system to support nucleic acid hybridization and microbiological growth
- Optical system comprised of a digital microscope, camera, and illuminator (both laser and darkfield light sources) for image capture of fluorescent signals and dark-field images
- Embedded control software, Graphical User Interface, and network connectivity
- Graphical data management, processing, analysis hardware, and software algorithms (designed to interpret images) in order to report identification and susceptibility results

The Accelerate PhenoTest BC kit contains a sample vial, a 48-channel disposable test cassette, and a reagent cartridge. Each module contains the electronics, motion control components, camera, illumination sources, and optical components necessary to process a patient sample.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ____X____ or No ________

Level of Concern:
Moderate

Software Description:
A detailed software description of the Accelerate Pheno system software, v1.0 was provided, which included a description of device features controlled by the software. A summary of the features also detailed the software operating environment (e.g. programming, language, hardware platform, operating system, use of off-the-shelf software).

The Accelerate Pheno system software is comprised of three (3) major architectural components:

Major Architectural Components	Function
Instrument Control	Responsible for execution of assay steps through modules that go through physical procedures and capture/deliver images for analysis. It also provides the user and LIS interfaces, management capabilities, and test result reporting for the modules
Module Firmware	Real-time control of hardware components inside a module (heaters, motors, fluidics, illumination, etc.) and communicates with instrument control software
Image Processing and Analysis	Analyzes images captured by the instrument to identify microbial species, quantify growth, and provide susceptibility results

Device Hazard Analysis
Risk analysis tables were provided that detailed software/hardware hazards related to the Accelerate Pheno system and the potential impact of each hazard on the performance of FISH ID and AST or possible injury to the user. The risk analysis listed each potential hazard, possible causes of the hazard, severity of the hazard, likelihood of occurrence, risk severity, and corrective measures. After applying mitigations, all potential risks were reduced to *"As Far as Possible"* or were considered *"Broadly Acceptable."*

For each hazard, identified lines provided software traceability to each Software Requirements Specification (including the Software Requirement Items) and Software Test Plan/Procedure. Mitigations included design, manufacturing controls, supplier management, product labeling, verification/validation and quality control testing.

Architecture Design Chart:
Diagrams of the system software architecture were provided to cover the Physical and Logical Architecture of the Accelerate Pheno system.

Software Requirements Specification (SRS) and Software Design Specification (SDS):
SRS documents were provided that described the requirements for various components of the Accelerate Pheno system. Each Software Requirement Item (SRI) specified the software requirement to be fulfilled for the product, including appropriate performance, function, reliability, and timing. New SRIs were assigned for new features, enhancements, and for any issue resolution. The SRS were under revision control and were updated with each newly assigned SRI. Requirements defined in the SRS document were implemented according to the design specifications described.

Traceability Analysis:
A traceability matrix that linked requirements, specifications, hazards, mitigations and verification & validation testing for the software was acceptable.

Software Development Environment Description:
The software development life cycle plan and software configuration management plan for the Accelerate Pheno system was acceptable.

Verification and Validation Testing (V&V):
Based on the SRS and SDS, software verification test plans were devised to verify that software met the requirements. Accelerate Pheno system software testing was successfully completed at unit, integration, and system levels. Pass/fail criteria were reported along with test results in the Software Validation Summary Reports.

Revision Level History:
The software revision history record for the Accelerate Pheno system software was acceptable.

Unresolved Anomalies:
A report of V&V testing throughout the software life cycle also included a list of all unresolved anomalies (as a result of V&V testing), known issues (before V&V testing), and resolved issues (after V&V testing). A risk analysis for each issue was performed, and results indicated that all remaining issues were those of low severity (Broadly Acceptable) and would not impact device performance. Any remaining issues, along with a work-around, will either be communicated to user through labeling or fixed in future software versions. The impact analyses of the unresolved anomalies on device safety and effectiveness were considered acceptable.

ES and EMC Testing:
The Accelerate Pheno system was tested and certified to the applicable electrical safety standards IEC 61010-1:2010 (3rd edition)—Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Part 1: General Requirements. The device also complied with electromagnetic compatibility, emission and immunity and the requirements of IEC 61326-2-6 and other applicable standards. Operational precautions, limitations hazards, and warnings were listed in the User Guide.

3. Specimen Identification:
   Sample vial can be labeled with barcode or written label. If the barcode is unreadable, the user can manually enter sample information to continue the run.

4. Specimen Sampling and Handling:
   The labeled Accelerate Pheno system sample vial is filled with an aliquot of positive blood culture. The sample vial cap is screwed on tightly. The test must be initiated within 15 minutes of placing the positive blood culture media in the Accelerate sample vial. A labeled sample vial containing positive blood culture is placed into the sample vial receptacle on the reagent cartridge, where the vial label is facing outward.

5. Calibration:
   There is no operator scheduled maintenance or operator replaceable parts or materials on the Accelerate Pheno system. An annual preventative maintenance visit by an authorized Accelerate Diagnostics service representative is recommended. Module relocation by the customer is not recommended. Relocation and recalibration should be performed by Accelerate trained service personnel before resuming normal operation. Contact Accelerate Diagnostics Technical Support to arrange for Accelerate trained service personnel to relocate the module.

6. Quality Control (QC):
   Three internal process controls are included in each assay:

   - Universal Bacterial Probe and Universal Eukaryotic Probe for yeast
   - General Nucleic Acid Stain
   - Growth Control Channel

   Instructions for QC testing with external controls are provided in the instructions for use. For QC panels used to monitor performance of ID and AST function of the Accelerate Pheno system, only one QC panel should be run on a given module at a time. Organisms for the QC panel being run should be inoculated into specified individual empty wells of the reagent cartridge. QC testing should be rotated between the modules such that all modules are used to perform QC at approximately the same frequency. External controls should be tested in accordance with the appropriate accrediting organization requirements.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

Q. Identified Risks to Health and Identified Mitigations

Identified Risks to Health	Identified Mitigations
If identification assay is included, false positive	General controls and special controls (1), (2),

or false negative results or incorrect identifications can lead to • a delay in determining the true cause of the infection • unnecessary, ineffective or lack of antimicrobial therapy • delayed or skipped treatments or diagnostic procedures • inappropriate infection prevention and control measures/and or public health procedures • interference with antimicrobial stewardship efforts Failure to perform appropriate AST testing may result in • unnecessary, ineffective or lack of antimicrobial therapy • interference with antimicrobial stewardship efforts • development of antimicrobial resistance An organism determined to be resistant when it is susceptible may lead to • treatment with an ineffective antibiotic • administration of unnecessary broad spectrum drugs • adverse effects from antimicrobials • costly implementation of infection control measures An organism determined to be susceptible when it is resistant may lead to • treatment with an ineffective antibiotic • increased morbidity or death	(3), (4), and (5)
Errors in Interpretation	General controls and special control (6)
Failure to correctly operate the test system	General controls and special control (7)

R. Benefit/Risk Analysis

<table>
<tr><th colspan="2">Summary</th></tr>
<tr><td>Summary of the Benefit(s)</td><td>The PhenoTest BC Kit is the first multiplexed in vitro diagnostic platform to use rapid nucleic acid fluorescence in situ hybridization (FISH) identification and quantitative AST to provide identification and susceptibility testing for sixteen pathogenic species of bacteria and yeast to aid in the diagnosis of bacteremia and fungemia.
The PhenoTest BC Kit returns results to patients and healthcare providers within six and a half hours of positive blood culture result, compared to current methods which may take 1-3 days to return results.
The PhenoTest BC Kit demonstrated PPA greater than 95%, NPA greater than 99%, and EA/CA greater than 90% for all microbial and drug targets.</td></tr>
<tr><td>Summary of the Risk(s)</td><td>False positive results, false negative results, misidentifications and incorrect AST results are the primary risks associated with use of the PhenoTest BC Kit.
A false positive result may lead to unnecessary antimicrobial therapy or incorrect antimicrobial therapy with subsequent delay in diagnosis. Patients may receive unnecessary infection control measures, such as contact isolation, if a highly resistant organism is thought to be present.
A false negative result may result in the inappropriate discontinuation or delay of antimicrobial therapy, with subsequent worsening of infection. Patients may also not receive sufficient infection control measures, such as contact isolation.
Misidentifications may occur if the device identifies the wrong microorganism and could result in the ineffective antimicrobial therapy and/or delay to appropriate therapy. Misidentifications could also result in incorrect infection control measures.
Errors in AST result could result in the wrong antibiotic choice with subsequent worsening of infection, or unnecessarily broad antibiotic treatment with the potential for the development of antimicrobial resistance or increased drug side effects.</td></tr>
<tr><td>Summary of Other Factors</td><td>None</td></tr>
<tr><td>Conclusions
Do the probable benefits outweigh the probable risks?</td><td>The probable benefits of the PhenoTest BC Kit outweigh the potential risks in light of the listed special controls and applicable general controls, including design controls. The PhenoTest BC Kit is the first of its kind and is likely to benefit patients through more timely identification and susceptibility testing of bloodstream infections, with the potential for improved patient outcomes and enhanced antimicrobial stewardship. The high performance observed during the pivotal clinical trials and the proposed special controls suggest that errors will be uncommon and will be well mitigated by laboratory practices and product labeling. The PhenoTest BC Kit could provide substantial benefits to the management of bloodstream infections as a complementary technology to current standard of care blood culture technology.</td></tr>
</table>

Patient Perspectives

This submission did not include specific information on patient perspective for this device.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.1650.

FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PRH, NSU, PEO, PAM, PEN, LON

Device Type: A cellular analysis system for multiplexed antimicrobial susceptibility testing

Class: II (special controls)

Regulation: 21 CFR 866.1650

(a) *Identification*. A cellular analysis system for multiplexed antimicrobial susceptibility testing is a multiplex qualitative and/or quantitative in vitro device intended for the identification and determination of the antimicrobial susceptibility results of organisms detected in samples from patients with suspected microbial infections. This device is intended to aid in the determination of antimicrobial susceptibility or resistance when used in conjunction with other laboratory findings.

(b) Classification. Class II (special controls). A cellular analysis system for multiplexed antimicrobial susceptibility testing must comply with the following special controls:

1) Premarket notification submissions must include detailed device description documentation, including the device components, ancillary reagents required but not provided, a detailed explanation of the methodology including primer/probe sequence, design, rationale for sequence selection and details of the antimicrobial agents, as applicable.

2) Premarket notification submissions must include detailed documentation from the following analytical and clinical performance studies: limit of detection, inclusivity, precision, reproducibility, interference, cross reactivity, carry-over, and cross contamination, quality control and additional studies as applicable to specimen type and assay claims.

3) Premarket notification submissions must include detailed documentation from an appropriate clinical study. The study, performed on a study population consistent with the intended use population, must compare the device performance to results obtained from well-accepted reference methods.

4) Premarket notification submissions must include detailed documentation for device software, including, but not limited to, software applications and hardware-based devices that incorporate software.

5) The 21 CFR 809.10(b) compliant labeling must include limitations and protocols regarding the need for correlation of results by standard laboratory procedures as applicable.

6) A detailed explanation of the interpretation of results and acceptance criteria must be included in the device's 21 CFR 809.10(b)(9) compliant labeling.

7) A detailed explanation of the principles of operation and procedures for assay performance and troubleshooting must be included in the device's 21 CFR 809.10(b) compliant labeling.