BIOMÉRIEUX

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BACT/ALERT® PF Plus

℞only IVD

Intended Use

BACT/ALERT® PF Plus culture bottles are used with BACT/ALERT® Microbial Detection Systems in qualitative procedures for recovery and detection of aerobic and facultative anaerobic microorganisms (bacteria and yeast) from blood.

Summary and Explanation

BACT/ALERT® Microbial Detection Systems are used to determine if microorganisms are present in blood taken from a patient suspected of having bacteremia/fungemia. The BACT/ALERT® System and culture bottles provide both a microbial detection system and a culture medium with suitable nutritional and environmental conditions for organisms commonly encountered in blood infections. The BACT/ALERT® PF Plus culture bottle provides for detection of microorganisms when a small volume of blood is available. An inoculated bottle is placed into the instrument where it is incubated and continuously monitored for the presence of microorganisms that will grow in the BACT/ALERT® PF Plus culture bottle.

Note: The information provided applies to all configurations of BACT/ALERT® Microbial Detection Systems, unless otherwise noted.

Principle of the Test

BACT/ALERT® Microbial Detection Systems utilize a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide is produced as the organisms metabolize the substrates in the culture medium. When growth of the microorganisms produces CO_2, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow.[1] The lighter color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes.

Reagents

For *in vitro* diagnostic use only.

Caution: Handle specimens and inoculated culture bottles as though capable of transmitting infectious agents. All inoculated culture bottles, specimen collection needles, and blood-drawing devices should be decontaminated according to your institution's procedures.[2]

BACT/ALERT® PF Plus (color-coded yellow) – BACT/ALERT® PF Plus disposable culture bottles contain 30 mL of complex medium and ≥1.6 g adsorbent polymeric beads. At time of manufacture, the medium consists of the following reactive components: combination of peptones/biological extracts (≥1.85% w/v), anticoagulant (≥0.083% w/v), vitamins and amino acids (≥0.00145% w/v), carbon sources (≥0.45% w/v), trace elements (≥0.0005% w/v) and other complex amino acid and carbohydrate substrates in purified water. Bottles contain an atmosphere of N_2, O_2, and CO_2, under vacuum. The composition of the medium may be adjusted to meet specific performance requirements and in such cases, analytical studies are conducted to establish substantial equivalence between the adjusted and previous formulations (refer to Revision History section).

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1 Thorpe TC, Wilson ML, Turner JE, et al. BacT/Alert: an Automated Colorimetric Microbial Detection System. *J Clin Micro* 1990; 28 (7), 1608-1612.

2 *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

Caution: BACT/ALERT® culture bottles contain polycarbonate. Not all disinfectants are intended for use with polycarbonate surfaces and may cause bottle deterioration. Verify disinfectant compatibility with polycarbonate before use on BACT/ALERT® culture bottle surfaces.

Caution: BACT/ALERT® PF Plus culture bottles used to culture very small blood specimen volumes (0.5 mL or less) will require added blood such as sterile defibrinated horse blood (10.0% w/v) to support growth, particularly for the recovery of fastidious organisms such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria gonorrhoeae*.[3]

Additional Materials Required

- BACT/ALERT® Microbial Detection Systems
- Blood-drawing device
- Sterile Airway Needle/Subculture Units
- Disposable gloves
- Appropriate biohazard waste containers for materials potentially contaminated with infectious agents
- Alcohol pads or equivalent

Materials Available from bioMérieux

- Blood Collection Adapter Cap
- BACT/ALERT® Microbial Detection Systems
- Sterile Airway Needle/Subculture Units

Storage Instructions

BACT/ALERT® PF Plus culture bottles are ready for use. Store in an upright position protected from direct light at room temperature (15-30°C). An expiration date is printed on each bottle label. Do not inoculate the culture bottles beyond the expiration date indicated. If the bottles are exposed to temperatures less than 15°C, precipitates may form that will disappear when the bottles are warmed to room temperature. Bottles must be at room temperature before use.

Chemical or Physical Indications of Instability

Prior to use, the BACT/ALERT® PF Plus culture bottles should be examined for evidence of damage or deterioration (discoloration). Bottles exhibiting evidence of damage, leakage, or deterioration should be discarded. The medium in undisturbed bottles should be clear, but there may be a slight opalescence or a trace of precipitate due to the anticoagulant SPS. Do not confuse opalescence with turbidity. Do not use a bottle which contains medium exhibiting turbidity, a yellow sensor, or excess gas pressure; these are signs of possible contamination.

Instruments

Review the appropriate BACT/ALERT® Microbial Detection System User Manual before use.

Specimen Collection and Preparation

General Considerations

1. BACT/ALERT® PF Plus culture bottles should be utilized by trained healthcare personnel. Correct specimen collection is extremely important when obtaining blood culture specimens. Refer to Cumitech 1C[3] for the proper specimen collection procedure.
2. Take care to prevent contamination during both bottle preparation and inoculation of the patient sample. Proper skin disinfection is an essential requirement to reduce the incidence of contamination.
3. Although not recommended by bioMérieux, blood may be drawn directly into collection tubes containing SPS. Tubes containing other anticoagulants should never be used for blood culture.[4]
4. bioMérieux recommends that inoculated culture bottles be placed into the BACT/ALERT® Microbial Detection System as soon as possible after collection. If there is an unavoidable delay, inoculated bottles may be maintained at room temperature up to 24 hours before loading into the instrument.

[3] Baron EJ, Weinstein MP, Dunne Jr. WM, Yagupsky P, Welch DF, Wilson DM. 2005. Cumitech 1C, Blood Cultures IV. Coordinating ed., Baron EJ. ASM Press, Washington, DC.

[4] CLSI. *Principles and Procedures for Blood Cultures*; Approved Guideline. CLSI document M47-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.

5. Optimal recovery of isolates will be achieved by adding maximum amounts of specimen. Use of lower volumes may adversely affect recovery and/or detection times of some organisms. The bottle's recommended specimen volume is up to 4 mL and the volume collected should be monitored by means of the 4 mL incremental markings on the bottle label.
6. In general, culturing higher volumes of blood containing small numbers of bacteria will improve recovery of bacteria by culture.[5]
7. The clinical studies were conducted with blood volumes as low as 0.1 mL. It is recommended, however, that up to 4 mL of blood be inoculated in the BACT/ALERT® PF Plus culture bottle. To prevent over inoculation, monitor the blood volume intake into the culture bottle, using the 4 mL incremental markings on the bottle label.

Caution: Direct vacuum draw using the BACT/ALERT® PF Plus culture bottle can result in the collection of greater than 4 mL blood sample volume. As a result, caution is advised when performing direct draw collection of blood from young children, infants, and neonates where total blood volume is a concern.

Bottle Preparation

1. Label the culture bottle with patient information. The icons on the bottle label (, #,) can be defined by the user.
2. Remove plastic flip-top from the culture bottle. Prior to inoculation, disinfect the culture bottle top with an alcohol swab or equivalent. Allow to air dry.
3. Clean the selected venipuncture site as recommended by your institution's approved procedure.

Venipuncture Direct Draw Inoculation Procedure

Note: If inoculating more than one type of BACT/ALERT® blood culture bottle using a butterfly blood collection set and direct draw adapter cap, inoculate first the aerobic culture bottle and then the anaerobic culture bottle so that any oxygen trapped in the tubing will not be transferred to the anaerobic bottle.

Note: Monitor the direct draw process closely at all times during collection to assure proper flow is obtained and to avoid flow of the bottle contents into the adapter tubing. Due to the presence of chemical additives in the culture bottle, it is important to prevent possible backflow and subsequent adverse reactions by following all steps below.

1. Hold the culture bottle at a position below the patient's arm with the bottle in an upright position (stopper uppermost).
2. Collect the blood using a butterfly blood collection set and a blood collection adapter cap as recommended by your institution's approved procedure and inoculate directly into the culture bottle at the patient's bedside. Although lower sample volumes can be used, recovery may be improved using a sample volume closer to the recommended 4 mL.
3. Release the tourniquet as soon as the blood starts to flow into the culture bottle, or within 2 minutes of application.
4. Do not allow the culture bottle contents to touch the stopper or the end of the needle during the collection procedure.

   Caution: A contaminated culture bottle could contain positive pressure, and if used for direct draw, may cause reflux into the patient's vein. Culture bottle contamination may not be readily apparent. Monitor the direct draw process closely to avoid reflux. Do not use a bottle that contains medium exhibiting turbidity, a yellow sensor, or excess gas pressure; these are signs of possible contamination.
5. Ensure that the specimen is properly mixed with the reagents in the BACT/ALERT® PF Plus bottle.
6. Transfer the inoculated culture bottle promptly to the testing laboratory.

Syringe Draw Inoculation Procedure

Note: If inoculating more than one type of BACT/ALERT® blood culture bottle using syringe draw, inoculate first the anaerobic culture bottle and then the aerobic culture bottle so that any oxygen trapped in the syringe will not be transferred to the anaerobic bottle. Line demarcations on the bottle label should be used to assist in estimating the sample volume.

1. Perform venipuncture and blood transfer to the BACT/ALERT® culture bottle according to your institution's established procedures.

   Caution: Never force the syringe plunger down during inoculation, as splashing of sample may occur. Remove the syringe when the fill amount is reached, as the vacuum will automatically draw more than the recommended maximum. Puncture the bottle stopper vertically to avoid releasing the vacuum; a bottle without a vacuum should not be inoculated.
2. Ensure that the specimen is properly mixed with the reagents in the BACT/ALERT® PF Plus bottle.
3. Transfer the inoculated culture bottle promptly to the testing laboratory.

[5] CLSI/NCCLS. *Quality Control for Commercially Prepared Microbiological Culture Media*; Approved Guideline. CLSI document M47-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.

BACT/ALERT® PF Plus Culture Bottle Test Procedure

Preliminary Comments and Precautions

1. Use disposable gloves and handle inoculated bottles cautiously as though capable of transmitting infectious agents. Consult a physician immediately if contaminated materials are ingested or come in contact with open lacerations, lesions, or other breaks in skin.
2. When handling positive bottles that are bulging or leaking, wear appropriate personal protective equipment (PPE) to avoid coming in contact with microorganisms.
3. Immediately clean up any spillage of contaminated material using a 1:10 dilution of 5% sodium hypochlorite. Dispose of the cleaning material by an acceptable method.
4. All inoculated culture bottles, specimen collection needles, and blood-drawing devices should be decontaminated according to your institution's procedures.[6]
5. These bottles should be utilized by trained healthcare personnel.

   Caution: For US Only: US Federal Law restricts this device to sale by or on the order of a licensed practitioner.

Procedural Notes and Precautions

1. Great care must be taken to prevent contamination of the patient sample during venipuncture and during inoculation into the culture bottle since contamination could lead to a specimen being determined positive when a clinically relevant isolate is not actually present.
2. Obtain blood samples prior to initiating antibiotic therapy. If this is not possible, draw blood immediately before administering the next antibiotic dose.
3. If inoculated culture bottles have been delayed in their receipt into the laboratory or have been incubated prior to entry into the BACT/ALERT® instrument, visually inspect for indications of microbial growth. If microbial growth is evident, treat the bottles as positive and do not place in the BACT/ALERT® Microbial Detection System for monitoring.

Laboratory Procedure

Caution: General caution should be taken when subculturing positive culture bottles as they could have been overfilled or contain high gas-producing organisms. Positive culture bottle contents may be under increased internal pressure. Positive culture bottles should be transiently vented before staining or disposal to release any gas produced during microbial metabolism.

1. Visually inspect bottles before testing. Do not use bottles with evidence of damage, leakage, or deterioration. Consider bottles with hemolysis, turbidity, excess gas pressure, yellow sensors, and/or evidence of growth as positive. Smear and subculture. Do not incubate unless smear is negative.
2. After culture bottles have been loaded into the instrument, incubate 5 days or until designated positive.
3. Smear and subculture all positive bottles. If the smear is negative, indicating a possible false positive, the bottle should be reloaded into the instrument until growth of the subculture or redesignation as positive. Bottles that were initially determined false positive and were redesignated positive should be smeared and subcultured.
4. Negative cultures may be checked by smear and/or subculture at some point prior to discarding as negative.
5. Procedures for loading and unloading culture bottles into the appropriate BACT/ALERT® instrument are given in the User Manual.
6. **Do not reuse BACT/ALERT® culture bottles.** Dispose of inoculated BACT/ALERT® culture bottles according to your laboratory protocol. Autoclaving and/or incinerating inoculated BACT/ALERT® bottles is appropriate.[7]
7. Utilization of coring devices (i.e., blunt needle) to puncture the septum may result in bottle leakage.

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[6] *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

[7] *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

Quality Control

A Certificate of Conformance is available for each lot of culture bottles. If desired, individual laboratories can perform quality control testing of BACT/ALERT® PF Plus culture bottles. Refer to the appropriate BACT/ALERT® User Manual and to CLSI® document M22-A3.[8]

Instrument

A BACT/ALERT® Reflectance Standards kit is provided with each BACT/ALERT® 3D instrument and Reflectance Calibration Standards are included with each BACT/ALERT® VIRTUO® instrument for the QC and Calibration procedures. All quality control should be part of normal system maintenance. Refer to the appropriate BACT/ALERT® User Manual for more information.

Caution: If your facility's LIS vendor sends bottle IDs and bottle type abbreviations to the BACT/ALERT® instrument, use the correct bottle type abbreviation to avoid possible false positive or false negative results. For more information, contact your local bioMérieux representative.

Results

Positive or negative culture bottles are determined by decision-making software contained in the BACT/ALERT® Microbial Detection Systems. No action is required until the BACT/ALERT® instrument signals culture bottles positive or negative.

Limitations of the Test

Many variables involved in blood culture testing cannot be practically controlled to provide total confidence that results obtained are due solely to proper or improper performance of any culture medium or detection system.

1. Patient specimens determined positive by BACT/ALERT® may contain organisms that are positive by smear that will not grow on routine subculturing media. When this is suspected, specimens should be subcultured on special media. Also, BACT/ALERT® positive specimens may contain organisms that are not seen with routine smear methods and may require both specialized smears and subculturing media for detection and recovery.
2. It is possible that certain rare, fastidious microorganisms will not grow or may grow slowly in the BACT/ALERT® PF Plus culture bottle growth medium. In addition, on rare occasions, organisms may be encountered that grow in the BACT/ALERT® PF Plus culture bottle growth medium but do not produce sufficient carbon dioxide to be determined positive. If rare, fastidious organisms requiring specialized media and culture conditions are suspected, alternative methods or extended incubation time should be considered for recovery.
3. Certain strains of *Haemophilus influenzae*, *Neisseria meningitidis*, and *Neisseria gonorrhoeae* may be sensitive to the anticoagulant SPS, which may result in a lack of growth or low production of CO_2 by these strains if an insufficient amount of sample is inoculated into the culture bottles.
4. Infrequently, if there is a very high number of white blood cells present in the sample, the BACT/ALERT® may indicate a culture bottle positive. In this case, the smear and subculture results may be negative.
5. Organisms are often few in numbers and may appear intermittently in the blood stream; therefore, several consecutive blood samples should be collected from each patient.
6. Promptly remove positive culture bottles when they are signaled by BACT/ALERT® to avoid possible non-viable cultures due to autolysis or other reasons. Certain strains of *Streptococcus pneumoniae* may be particularly prone to autolysis if they are not removed promptly after being signaled positive.
7. A Gram-stained smear from a negative bottle may sometimes contain a small number of non-viable organisms that were derived from culture medium components, staining reagents, immersion oil, or glass slides, resulting in a false positive smear.
8. bioMérieux recommends that inoculated culture bottles be placed into the BACT/ALERT® Microbial Detection System as soon as possible after collection. But, in the unavoidable cases when there is a delay in bottle receipt by the laboratory, delayed entry information is provided from seeded studies in the "Performance Characteristics of the Test" section.
9. Antimicrobial neutralization was not achieved for ceftazidime or cefepime.

[8] CLSI®/NCCLS. *Quality Control for Commercially Prepared Microbiological Culture Media*; Approved Standard—Third Edition. CLSI®/NCCLS document M22-A3. Wayne, PA: NCCLS; 2004.

Expected Values

1. Percent positive cultures were observed to be 6.3% (range: 4.9%-8.1%) overall and 4.1% (range: 2.5%-6.4%) for significant isolates from three clinical trial sites in BACT/ALERT® PF Plus culture bottles that received 0.1-4 mL of blood during the BACT/ALERT® PF Plus culture bottle clinical trial conducted with the BACT/ALERT® 3D.
2. For BACT/ALERT® 3D testing during the BACT/ALERT® VIRTUO® clinical trial, percent positive cultures were observed to be 1.5% (range: 0.0%-11.1%) overall and 1.5% (range: 0.0%-11.1%) for significant isolates from three clinical trial sites in BACT/ALERT® PF Plus culture bottles that received ≤4 mL of blood.
3. For BACT/ALERT® 3D testing during the BACT/ALERT® VIRTUO® clinical trial, percent positive cultures were observed to be 8.4% (range: 3.6%-13.6%) overall and 6.9% (range: 3.6%-13.6%) for significant isolates from three clinical trial sites in BACT/ALERT® FA Plus culture bottles that received ≤4 mL of blood.
4. For the BACT/ALERT® VIRTUO® clinical trial, percent positive cultures were observed to be 2.0% (range: 0.0%-2.1%) overall and 1.5% (range: 0.0%-1.6%) for significant isolates from three clinical trial sites in BACT/ALERT® PF Plus culture bottles that received ≤4 mL of blood.
5. For the BACT/ALERT® VIRTUO® clinical trial, percent positive cultures were observed to be 8.2% (range: 6.9%-13.6%) overall and 6.9% (range: 5.7%-12.5%) for significant isolates from three clinical trial sites in BACT/ALERT® FA Plus culture bottles that received ≤4 mL of blood.
6. Expected percent positives will vary based on factors such as patient population, prevalence of significant organisms, site location, and contamination rates. The expected values provided are based on clinical study data acquired during multiple clinical studies.

Neutralization of Antimicrobials

Neutralization of antimicrobials by adsorbent polymeric beads varies depending upon dosage level and timing of specimen collection. Internal studies tested on the BACT/ALERT® 3D Microbial Detection System have demonstrated that antimicrobials are effectively neutralized by the BACT/ALERT® PF Plus medium based on 100% recovery of the organisms tested. In these tests, antimicrobials were added in clinically relevant concentrations directly to culture bottles during inoculation with susceptible strains. The effectiveness of the antimicrobials was confirmed by parallel testing using a non-neutralizing medium as a control. Antimicrobials from the following categories were neutralized by the medium: penicillins, glycylcyclines, polyenes, macrolides, triazoles, echinocandins, cefazolin, cefoxitin, ceftaroline, aminoglycosides, fluoroquinolones, lincosamides, glycopeptides, and oxazolidinones.

Antimicrobial neutralization was not achieved for ceftazidime or cefepime. Less than complete neutralization was observed for cefotaxime and ceftriaxone. Cefotaxime was neutralized at ranges of 50% peak serum level (PSL) to 2% PSL depending on the microorganism. Ceftriaxone was neutralized at ranges of 50% PSL to 1% PSL depending on the organism.

Antimicrobial neutralization properties are dependent upon the culture bottle material composition and are not determined by the analysis algorithms of the BACT/ALERT® Microbial Detection Systems. Representative antimicrobials selected from four of the categories listed above were tested on the BACT/ALERT® VIRTUO® Microbial Detection System to confirm neutralization properties of the BACT/ALERT® PF Plus culture bottles. Neutralization was demonstrated for amikacin (from the aminoglycosides class), piperacillin (penicillins class), vancomycin (glycopeptides class), and voriconazole (triazoles class). Testing demonstrated that the instrument system had no impact on culture bottle antimicrobial neutralization properties.

After formulation adjustments, representative antimicrobials from the claimed drug categories were tested in the adjusted BACT/ALERT® PF Plus culture bottle in the absence of blood, unless otherwise noted, on both the BACT/ALERT® 3D and BACT/ALERT® VIRTUO® Microbial Detection Systems. Antimicrobials from the following categories were neutralized by the adjusted medium: penicillins, glycylcyclines, polyenes, macrolides, triazoles, echinocandins, aminoglycosides, fluoroquinolones, lincosamides, glycopeptides, and oxazolidinones. Five echinocandin/microorganism combinations were tested; one combination had a recovery rate of less than 100%. In the absence of blood, a 77.8% recovery rate was observed for *C. albicans*/caspofungin in the adjusted BACT/ALERT® PF Plus culture bottle. In the presence of blood, a 97% recovery rate was observed in the adjusted BACT/ALERT® PF Plus for *C. albicans*/caspofungin. In addition, substantial equivalency for ceftaroline, cefoxitin, and cefazolin was observed between the adjusted and previous BACT/ALERT® PF Plus culture bottles for neutralization of the individual drugs. For *S. aureus*/cefoxitin, a 66% recovery was observed initially; however, retest results showed 100% recovery. For *E.coli*/cefoxitin, a 100% recovery was observed. Less than complete neutralization was observed for cefazolin, which was neutralized at 50% peak serum level (PSL) when tested in PBS.

Ceftazidime, cefepime, cefotaxime, and ceftriaxone were not evaluated in the adjusted BACT/ALERT® PF Plus culture bottle as less than complete neutralization was achieved in the previous formulation.

For additional information on antimicrobial agents neutralized by BACT/ALERT® PF Plus culture bottles, contact your local bioMérieux representative.

Performance Characteristics

BACT/ALERT® 3D Microbial Detection Systems

Potentially Interfering Substances
In-house seeded studies were conducted with plasma, blood, and blood clots. Aliquots of each of these fluids also received white blood cells at concentrations relevant to bacteremia in blood. Testing was conducted with and without microorganisms. These substances neither interfered with recovery and detection of organisms, nor did they generate false positive results in the absence of organisms.

Analytical Sensitivity: Limit of Detection (LoD)
Data in the following table represent results from in-house seeded studies. A minimum of 30 replicates were tested per species. At least 95% detection was achieved at LoD. Data in the following table were generated using bottles at the end of shelf life. Bottles inoculated with *H. influenzae* received 4 mL pooled human blood supplementation.

Table 1: Analytical Sensitivity: Limit of Detection (LoD)

Microorganism	Strain ID	LoD (CFU/bottle)
Candida albicans	ATCC® 14053™	6
Enterobacter aerogenes	ATCC® 13048™	8
Enterococcus faecalis	NCTC 12697	5
Escherichia coli	NCTC 12923	4
Haemophilus influenzae	ATCC® 10211™	6
Klebsiella pneumoniae	STL 104016	4
Listeria monocytogenes	ATCC® 15313™	6
Pseudomonas aeruginosa	NCTC 12924	4
Salmonella enterica	ATCC® 14028™	5
Staphylococcus aureus	NCTC 10788	5
Streptococcus pneumoniae	ATCC® 6305™	6

Note: 96.7% of the bottles were subcultured within 30 minutes of being declared positive. STL 104016 was sourced from bioMérieux's internal culture collection.

Analytical Sensitivity: Growth Performance
Data in the following table represent results from in-house seeded studies with and without blood obtained from healthy human volunteers. Multiple strains were tested for each species at target inoculum levels of 125 CFU/bottle. The actual inoculum levels ranged from 3-298 CFU/bottle. In this seeded study, the BACT/ALERT® PF Plus culture bottles were subcultured at least 24 hours after being flagged positive by the instrument. The species listed are representatives of clinically prevalent organisms in blood cultures.

Table 2: Analytical Sensitivity: Growth Performance

Microorganism	Blood			
	% Recovery(n)	Range (CFU/Bottle)	Time to Detection (hours)	
			Mean	Range
Staphylococcus aureus	100.0 (30/30)	54-150	13.3	12.2-15.2
Escherichia coli	100.0 (30/30)	71-254	11.2	10.3-11.7
Pseudomonas aeruginosa	100.0 (12/12)	74-148	15.7	13.7-17.8
Klebsiella pneumoniae	100.0 (12/12)	89-123	11.3	10.6-12.3
Candida albicans	100.0 (30/30)	88-298	29.0	19.2-52.8
Streptococcus pneumoniae	100.0 (30/30)	3-260	13.8	10.8-16.5
Staphylococcus epidermidis	100.0 (12/12)	44-135	17.6	14.3-18.8
Enterococcus faecalis	100.0 (12/12)	63-259	11.6	11.0-12.2
Enterococcus faecium	100.0 (12/12)	25-120	12.8	11.3-14.4
Enterobacter cloacae	100.0 (12/12)	111-200	11.6	10.8-12.5
Candida glabrata	100.0 (12/12)	118-281	43.5	27.3-64.8
Haemophilus influenzae	100.0 (12/12)	105-266	14.4	12.0-16.8
Proteus mirabilis	100.0 (12/12)	36-213	12.5	11.3-14.6

Less than 100% detection was observed for some species, including *Capnocytophaga ochracea*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Haemophilus parainfluenzae*, *Granulicatella adiacens*, and *Helicobacter cinaedi*.

After formulation adjustments, a direct comparison study was conducted with the adjusted and previous BACT/ALERT® PF Plus culture bottles using a panel of clinically relevant microorganisms tested in the presence and absence of blood on both the BACT/ALERT® 3D and BACT/ALERT® VIRTUO® Microbial Detection Systems. The recovery rate for the 39 microorganisms evaluated for growth performance in the adjusted BACT/ALERT® PF Plus culture bottle met the criteria for equivalency to the previous BACT/ALERT® PF Plus culture bottle.

The time-to-detection (TTD) for 38 of the 39 microorganisms evaluated for growth performance in the adjusted BACT/ALERT® PF Plus culture bottle in the presence of blood met the criteria for equivalency to the previous BACT/ALERT® PF Plus culture bottle. A delay in TTD was observed for *Haemophilus parainfluenzae* in the adjusted BACT/ALERT® PF Plus culture bottle, although both the adjusted and previous had less than 100% detection, as noted under Table 2. In the presence of 4 mL blood, a faster TTD was achieved for *C. glabrata* with a mean TTD improvement of 30 hours.

Clinical Study Results (Blood Cultures)
Results compare BACT/ALERT® PF Plus to BACT/ALERT® PF blood cultures (for all compliant pairs).

A multi-center clinical study was conducted at three different geographic sites in the U.S. comparing the performance of the BACT/ALERT® PF Plus and BACT/ALERT® PF blood culture bottles for pediatric culture pairs that received blood volumes between 0.1 mL and 4 mL (compliant pairs). A total of 2188 bottle pairs were obtained from 1086 pediatric patients suspected of blood stream bacterial/yeast infections. Subcultures of both bottles were performed when either bottle in the set was determined to be positive by the BACT/ALERT® System. A pair of bottles was determined to have a positive status if the subculture of either the BACT/ALERT® PF Plus or BACT/ALERT® PF culture bottle was positive. A culture bottle was determined to be a "True Positive" if the culture was flagged positive by the BACT/ALERT® System and resulted in growth of the isolate upon subculture of this bottle. True positive rates were calculated for the BACT/ALERT® PF Plus and BACT/ALERT® PF culture bottles, and the ratio of BACT/ALERT® PF Plus true positives to BACT/ALERT® PF true positives was calculated to compare performance. Clinical isolates recovered were classified as significant, contaminant, or unknown based on determination by the clinical trial sites.

A total of 172 isolates were recovered from all compliant pediatric blood culture pairs with a positive status. There were a total of 145 bottle pairs that recovered at least one isolate by subculture of BACT/ALERT® PF Plus or BACT/ALERT® PF culture bottles. A total of 126 bottle pairs recovered a single isolate, 12 bottle pairs recovered two isolates, six bottle pairs recovered three isolates, and one bottle pair recovered four isolates. The total population reported in Tables 3, 4, and 5 comprises the 172 isolates recovered from positive bottle pairs and 2043 negative bottle pairs for a total of 2215 results. The BACT/ALERT® PF Plus culture bottle detected a total of 140 isolates compared to the BACT/ALERT® PF culture bottle that

detected 128 isolates. Of the significant isolates, the BACT/ALERT® PF Plus culture bottle detected a total of 91 isolates compared to the BACT/ALERT® PF culture bottle that detected 77 isolates. One false positive was identified by subculture of a positive BACT/ALERT® PF Plus culture bottle and comprised 0.05% (1/2215) of the study population.

The following tables compare results of the BACT/ALERT® PF Plus to BACT/ALERT® PF blood cultures for all compliant blood culture bottles that yielded any number of isolates on subculture (Table 3), a single isolate alone on subculture (Table 4), and multiple isolates on subculture (Table 5).

Table 3: All Compliant Pairs with Single and Multiple Isolates Combined (Blood Cultures)

Clinical Isolate Determination	BACT/ALERT® PF Plus True Positives	% of BACT/ALERT® PF Plus True Positives in Population	BACT/ALERT® PF True Positives	% of BACT/ALERT® PF True Positives in Population	Ratio of True Positives*
Significant	91	4.1 (91/2215)	77	3.5 (77/2215)	1.182
Contaminant	24	1.1 (24/2215)	29	1.3 (29/2215)	0.828
Unknown	25	1.1 (25/2215)	22	1.0 (22/2215)	1.136
Total	140	6.3 (140/2215)	128	5.8 (128/2215)	1.094

*Ninety-six (96) isolates were detected by both the BACT/ALERT® PF Plus and BACT/ALERT® PF, 44 isolates were detected only by BACT/ALERT® PF Plus, and 32 isolates were detected only by BACT/ALERT® PF. The ratio of true positive rates for overall isolates was 1.094 (140/128) with a 95% CI (0.954, 1.234). [7]

Table 4: All Compliant Pairs with Single Isolates (Blood Cultures)

Clinical Determination	BACT/ALERT® PF Plus True Positives	BACT/ALERT® PF True Positives	Ratio of True Positives*
Significant	69	61	1.131
Contaminant	17	17	1.000
Unknown	19	16	1.188
Total	105	94	1.117

*Seventy-three (73) isolates were detected by both the BACT/ALERT® PF Plus and BACT/ALERT® PF, 32 isolates were detected only by BACT/ALERT® PF Plus, and 21 isolates were detected only by BACT/ALERT® PF. The ratio of true positive rates for overall single isolates was 1.117 (105/94) with a 95% CI (0.957, 1.277). [7]

Table 5: All Compliant Pairs with Multiple Isolates (Blood Cultures)

Clinical Determination	BACT/ALERT® PF Plus True Positives	BACT/ALERT® PF True Positives	Ratio of True Positives*
Significant	22	16	1.375
Contaminant	7	12	0.583
Unknown	6	6	1.000
Total	35	34	1.029

*Twenty-three (23) isolates were detected by both the BACT/ALERT® PF Plus and BACT/ALERT® PF, 12 isolates were detected only by BACT/ALERT® PF Plus, and 11 isolates were detected only by BACT/ALERT® PF. The ratio of true positive rates for overall multiple isolates was 1.029 (35/34) with a 95% CI (0.748, 1.310). [7]

In this clinical study, there were a total of 2041 pairs of BACT/ALERT® PF Plus and BACT/ALERT® PF culture bottles with negative instrument results for both bottles after 5 days of incubation. Among these pairs, terminal subcultures on both bottles were performed for three pairs, and no false negative results by both BACT/ALERT® PF Plus and BACT/ALERT® PF culture bottles were observed; subculture on BACT/ALERT® PF Plus culture bottles alone was performed for 2034 pairs, and one false negative result was observed; both subcultures were not performed for four pairs of bottles. Results are summarized in the following table.

Table 6: Summary of Percent False Negatives from Aerobic Blood Culture Pairs That Were Flagged Negative by Both Instruments

Subculture Performed BACT/ALERT® PF Plus	Subculture Performed BACT/ALERT® PF	% False Negative BACT/ALERT® PF Plus	% False Negative BACT/ALERT® PF
Yes	Yes	0.0 (0/3)	0.0 (0/3)
Yes	No	0.05 (1/2034)	-

Overall false negative rate for BACT/ALERT® PF Plus based on a subset of terminal subcultures was 0.05% (1/2037).

A comparative yield of microorganisms (number of isolates) recovered on subculture of BACT/ALERT® PF Plus and BACT/ALERT® PF cultures is presented in the following table.

Table 7: Comparative Yield of Microorganisms (Number of Isolates) – Blood Cultures

Microorganism Group	Pediatric Subgroup	BACT/ALERT® PF Plus	BACT/ALERT® PF Plus Fill Range (mL)	BACT/ALERT® PF	BACT/ALERT® PF Fill Range (mL)
Enterobacteriaceae	Newborn (< 1 mo)	6	0.1-1.4	7	0.1-1.6
	Infant (> 1 mo-2 yrs)	19	0.1-3.7	13	0.1-3.1
	Child (> 2 yrs-12 yrs)	9	0.6-3.9	7	0.3-3.2
	Adolescent (> 12 yrs-21 yrs)	0	1.4	1	1.6
Fastidious (*Neisseria meningitidis*, *Neisseria sicca*)	Newborn (< 1 mo)	0	-	0	-
	Infant (> 1 mo-2 yrs)	1	0.2	1	0.5
	Child (> 2 yrs-12 yrs)	0	-	0	-
	Adolescent (> 12 yrs-21 yrs)	1	1.1	1	0.6
Yeast (*Candida albicans*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*)	Newborn (< 1 mo)	0	-	0	-
	Infant (> 1 mo-2 yrs)	0	0.5	1	0.9
	Child (> 2 yrs-12 yrs)	5	0.9-3.7	6	1.0-3.4
	Adolescent (> 12 yrs-21 yrs)	1	0.2-3.0	2	2.1-2.5
Non-fermentative Gram-Negative Bacilli	Newborn (< 1 mo)	0	-	0	-
	Infant (> 1 mo-2 yrs)	5	1.7-3.5	6	1.5-2.5
	Child (> 2 yrs-12 yrs)	2	0.9-2.2	3	1.0-2.8
	Adolescent (> 12 yrs-21 yrs)	0	-	0	-

Microorganism Group	Pediatric Subgroup	BACT/ALERT® PF Plus	BACT/ALERT® PF Plus Fill Range (mL)	BACT/ALERT® PF	BACT/ALERT® PF Fill Range (mL)
Coagulase-Negative *Staphylococcus*	Newborn (< 1 mo)	5	0.1-0.5	5	0.1-0.9
	Infant (> 1 mo-2 yrs)	12	0.1-3.0	10	0.1-3.4
	Child (> 2 yrs-12 yrs)	15	0.1-3.8	12	0.5-3.6
	Adolescent (> 12 yrs-21 yrs)	6	0.5-3.5	7	0.5-3.2
Staphylococcus aureus	Newborn (< 1 mo)	0	0.3	1	0.1
	Infant (> 1 mo-2 yrs)	5	0.5-1.5	5	0.6-1.6
	Child (> 2 yrs-12 yrs)	7	0.8-4.0	3	0.1-3.6
	Adolescent (> 12 yrs-21 yrs)	2	1.5-1.7	2	1.3-1.4
Enterococcus spp.	Newborn (< 1 mo)	1	0.1	1	0.1
	Infant (> 1 mo-2 yrs)	9	0.2-2.9	10	0.1-3.2
	Child (> 2 yrs-12 yrs)	2	0.2-1.0	1	0.8-1.8
	Adolescent (> 12 yrs-21 yrs)	8	1.5-3.1	7	1.9-2.9
Streptococcus pneumoniae	Newborn (< 1 mo)	0	-	0	-
	Infant (> 1 mo-2 yrs)	2	1.0-2.7	1	1.1-1.6
	Child (> 2 yrs-12 yrs)	0	-	0	-
	Adolescent (> 12 yrs-21 yrs)	0	-	0	-
Streptococcus spp., Group A, B	Newborn (< 1 mo)	0	-	0	-
	Infant (> 1 mo-2 yrs)	1	0.5	0	1.0
	Child (> 2 yrs-12 yrs)	0	-	0	-
	Adolescent (> 12 yrs-21 yrs)	0	-	0	-
Other *Streptococcus* spp.	Newborn (< 1 mo)	0	-	0	-
	Infant (> 1 mo-2 yrs)	4	0.1-1.6	5	0.1-1.5
	Child (> 2 yrs-12 yrs)	3	0.7-2.3	2	0.6-2.6
	Adolescent (> 12 yrs-21 yrs)	1	0.7-2.4	2	1.1-2.4

Microorganism Group	Pediatric Subgroup	BACT/ALERT® PF Plus	BACT/ALERT® PF Plus Fill Range (mL)	BACT/ALERT® PF	BACT/ALERT® PF Fill Range (mL)
Other Gram-Negative*	Newborn (< 1 mo)	0	-	0	-
	Infant (> 1 mo-2 yrs)	1	0.6	0	0.4
	Child (> 2 yrs-12 yrs)	0	-	0	-
	Adolescent (> 12 yrs-21 yrs)	0	-	0	-
Other Gram Positive†	Newborn (< 1 mo)	1	0.1-0.7	2	0.1-0.4
	Infant (> 1 mo-2 yrs)	3	0.5-3.0	2	0.9-2.3
	Child (> 2 yrs-12 yrs)	3	0.1-3.4	1	0.1-3.2
	Adolescent (> 12 yrs-21 yrs)	0	3.3	1	3.2

* Other Gram-Negative Organisms recovered in clinical study: Unidentified Gram-Negative Rods (1)

† Other Gram-Positive Organisms recovered in clinical study: *Bacillus* spp. (6), *Corynebacterium* spp. (2), Diphtheroids (1), *Micrococcus* spp. (2), *Stomatococcus* spp. (1).

Organisms were recovered in the clinical studies at blood volumes greater than 0.1 mL. They are:
- ≥1.0 mL for *Streptococcus pneumoniae*
- ≥0.9 mL for non-fermenting Gram-Negative Rods; ≥0.6 for unidentified Gram-Negative Rods
- ≥0.5 mL for Group A, B *Streptococcus* spp.
- ≥0.3 mL for *S. aureus*
- ≥0.2 mL for fastidious organism (*N. meningitidis* and *N. sicca*)
- ≥0.2 mL for Yeast (*Candida albicans*, *C. guilliermondii*, *C. krusei*, and *C. lusitaniae*)

Quality control was performed during the clinical study on each of the 13 organisms (*Candida albicans*, *Candida krusei*, *Enterococcus faecalis*, *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*), which were prepared using serial dilution and seeded into the BACT/ALERT® PF Plus culture bottle at a target inoculum of 100 CFU/bottle, with an acceptable range of 30-300 CFU/bottle. Overall quality control results were found to be acceptable. Instances where unacceptable quality control results were observed were found to be due to technical errors (i.e., colony counts out of range, site failure to change bottle status after positive instrument signal and positive subculture, and no supplement added). Repeat testing resulted in acceptable results.

Delayed Entry
The following table includes results from seeded studies using 11 species (*Staphylococcus aureus*, *Candida albicans*, *Candida krusei*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Enterococcus faecium*, *Haemophilus influenzae*, and *Neisseria meningitidis*), at target concentrations of 100 CFU/bottle (acceptable range of 30-300 CFU/bottle) were generated at three sites. Actual inoculum levels ranged from 35-290 CFU/bottle, although there was an instance of a colony count that was >300 CFU/bottle which was not included in the final data analysis. Bottles were tested with human blood from healthy volunteers and were held at specified temperatures and times prior to loading into the BACT/ALERT® 3D instrument. Percent recovery reflects bottles flagged positive by the instrument and subculture with colony morphology consistent with the seeded organism.

Table 8: Delayed Entry

Sample Input	Incubation Temperature (°C)	Hold Time (hours)	% Recovery	Time to Detection from Sample Inoculation (Hold Time + Instrument TTD in hours)		Inoculum Range (CFU/bottle)
				Mean	Range	
Inoculated Test Bottles	Control	No delay	100.0 (459/459)	14.3	8.5-84.0	35-288
	2-8	48	98.6 (292/296)	63.7	57.5-103.2	48-288
	20-25	24	98.0 (291/297)	31.8	26.2-74.4	50-288
	20-25	36	91.9 (272/296)	41.8	38.0-70.5	50-290
	35-37	8	98.9 (454/459)	16.1	10.2-53.8	35-288
	35-37	24	56.6 (259/458)	28.3	26.0-74.4	35-288
Negative Controls	All conditions		0.5 (1/221)*	-	-	-

* One false positive result observed during seeded study. Negative confirmation by Gram stain/subculture.

Important: Culture bottles held at 35-37°C for 24 hours or longer before loading may not detect microorganisms and should be subcultured.

Within-Laboratory Precision (Repeatability)
Data in the following table represent results from in-house seeded studies conducted on 12 days on multiple instruments by multiple operators. Organisms were grown in the presence of clinically relevant concentrations of antimicrobials to which they are susceptible. In this seeded study BACT/ALERT® PF Plus culture bottles were subcultured at least 24 hours after being flagged positive by the instrument. A minimum of 108 replicates were tested for each organism/antimicrobial combination.

Table 9: Within-Laboratory Precision (Repeatability)

Sample Input		Range (CFU/bottle)	% Recovery				Time to Detection (hours)	
Microorganism	Antimicrobial		Lot 1	Lot 2	Lot 3	Overall	Mean	Range
C. albicans	Fluconazole	140-364	100.0	100.0	100.0	100.0	26.0	22.8-31.3
E. coli	Amikacin	26-156	100.0	100.0	100.0	100.0	12.0	11.2-13.0
K. pneumoniae	Levofloxacin	108-170	100.0	100.0	100.0	100.0	13.4	11.7-15.2
P. aeruginosa	Piperacillin	80-148	100.0	97.2	100.0	99.1	19.2	17.4-24.1
S. pneumoniae	Penicillin G	9-505	100.0	100.0	100.0	100.0	13.2	11.6-15.5
S. aureus	Vancomycin	94-158	100.0	100.0	100.0	100.0	16.9	14.6-20.3

Reproducibility
Data in the following table represent results from seeded studies conducted at three sites using a target of 162 replicates per site on 3 days with a minimum of two operators per site. Reproducibility was evaluated on each of nine organisms. Two organisms (*C. albicans* and *S. pneumoniae*) were prepared using serial dilution and the other seven organisms were prepared using BIOBALL® products. *C. albicans* and *S. pneumoniae* were seeded into the BACT/ALERT® PF Plus culture bottle, at a target inoculum of 100 CFU/bottle, with an acceptable range of 30-300 CFU/bottle and the other seven organisms at a target range of 1-17 CFU/bottle. The actual inoculum ranged from 6-700 CFU/bottle for the 30-300 CFU/bottle range, and from 1-270 CFU/bottle for the 1-17 CFU/bottle range. Percent recovery reflects positive flag by the instrument and Gram-stain/subculture consistent with the seeded organism.

Table 10: Reproducibility

Sample Input	% Recovery				Time to Detection (hours)		Inoculum Ranges (CFU/Bottle)
	Site 1	Site 2	Site 3	Overall	Mean	Range	
S. aureus	100.0% (18/18)	87.5% (21/24)	100.0% (30/30)	95.8% (69/72)	15.6	14.6-16.7	2-11
C. albicans	100.0% (18/18)	83.3% (30/36)	100.0% (33/33)	93.1% (81/87)	36.6	24.6-76.8	14-700
E. coli	100.0% (27/27)	77.8% (21/27)	100.0% (30/30)	92.9% (78/84)	12.8	11.8-14.1	1-38
P. aeruginosa	100.0% (24/24)	75.0% (18/24)	97.0% (32/33)	91.4% (74/81)	18.4	17.1-21.1	1-11
E. faecalis	100.0% (18/18)	79.2% (19/24)	96.7% (29/30)	91.7% (66/72)	13.9	12.6-15.3	1-15
E. aerogenes	74.4% (29/39)	72.2% (26/36)	85.4% (41/48)	78.1% (96/123)	14.9	11.7-20.8	<1-270*
L. monocytogenes	100.0% (18/18)	100.0% (24/24)	100.0% (30/30)	100.0% (72/72)	24.1	20.4-36.4	1-14
S. enterica	100.0% (24/24)	75.0% (18/24)	100.0% (33/33)	92.6% (75/81)	13.5	2.3-14.8	1-13
S. pneumoniae	100.0% (30/30)	100.0% (36/36)	100.0% (21/21)	100.0% (87/87)	14.2	11.6-18.9	6-500
Overall	95.4% (206/216)	83.5% (213/255)	96.9% (279/288)	92.0% (698/759)	-		
	95% CI: 91.7%, 97.8%	95% CI: 78.4%, 87.9%	95% CI: 94.2%, 98.6%	95% CI: 89.8%, 93.8%			

* Plate count of 270 CFU/bottle was arrived at by serial dilution.

These data include repeat testing performed as a result of laboratory errors at a single site (i.e., contaminated subculture). Data excluding the laboratory errors demonstrated 100% recovery with the exception of *E. aerogenes*, which exhibited 85% recovery for all sites combined.

BACT/ALERT® VIRTUO® Microbial Detection Systems

Potentially Interfering Substances
In-house seeded studies were conducted with plasma, blood, and blood clots. Aliquots of each of these fluids also received white blood cells at concentrations relevant to bacteremia in blood. Testing was conducted with and without microorganisms. These substances neither interfered with recovery and detection of organisms, nor did they generate false positive results in the absence of organisms.

Analytical Sensitivity: Limit of Detection (LoD)
Data in the following table represent results from in-house seeded studies. A minimum of 60 BACT/ALERT® PF Plus culture bottles were tested per species. At least 95% detection was achieved at LoD. Bottles inoculated with *H. influenzae* received 1 mL of human blood obtained from a healthy adult population.

Table 11: Analytical Sensitivity: Limit of Detection (LoD)

Microorganism	Strain ID	BACT/ALERT® VIRTUO® (CFU/bottle)
Candida albicans	ATCC® 14053™	7
Enterococcus faecalis	NCTC 12697	4
Escherichia coli	NCTC 12923	4
Haemophilus influenzae	ATCC® 10211™	3
Pseudomonas aeruginosa	ATCC® 9027™	8
Staphylococcus aureus	NCTC 10788	3
Streptococcus pneumoniae	ATCC® 6305™	3

Delayed Entry
The following table includes results from seeded studies using 9 species (*Staphylococcus aureus*, *Candida albicans*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, and *Enterococcus faecium*) at target concentrations of ≤100 CFU/bottle. Actual inoculum levels ranged from 5-84 CFU/bottle. Bottles were tested without and with 4 mL and 10 mL human blood from healthy volunteers and were held at specified temperatures and times prior to loading into the BACT/ALERT® VIRTUO® instrument. Percent recovery reflects bottles flagged positive by the instrument and subculture with colony morphology consistent with the seeded organism. Negative controls were tested with 10 mL human blood as a more stringent test to evaluate the risk of false positives.

Table 12: Delayed Entry

Sample Input	Incubation Temperature (°C)	Hold Time (hours)	% Recovery	Time to Detection from Sample Inoculation (Hold Time + Instrument TTD in hours)	
				Mean	Range
Inoculated Test Bottles	Control	No delay	100.0 (259/259)	14.0	8.5-30.0
	35-37	8	100.0 (261/261)	16.5	10.9-31.1
	20-25	24	100.0 (261/261)	32.1	27.6-46.6
	20-25	36	99.6 (260/261)	42.0	37.7-65.1
	2-8	48	96.5 (247/256)*	61.3	57.2-77.9
Negative Controls	All conditions		0.0 (0/64)†	-	-

* 8/9 negative bottles were observed when tested with *N. meningitidis*.

† Negative controls tested with 10 mL human blood.

Caution: Culture bottles held at room temperature for longer than 24 hours before loading may not detect microorganisms and should be subcultured.

Within-Laboratory Precision (Repeatability)
Data in the following table represent results from in-house seeded studies conducted for 20 days using multiple instruments and tested by multiple operators. A minimum of 60 replicates were tested for each organism and lot tested.

Table 13: Within-Laboratory Precision (Repeatability)

Microorganism	Range (CFU/bottle)	% Recovery				Time to Detection (hours)	
		Lot 1	Lot 2	Lot 3	Overall	Mean	Range
Candida albicans	3-30	100.0 (141/141)	100.0 (144/144)	100.0 (60/60)	100.0 (345/345)	27.1	21.4-40.0
Enterococcus faecalis	3-16	100.0 (162/162)	100.0 (162/162)	100.0 (60/60)	100.0 (384/384)	10.9	9.6-12.4
Escherichia coli	8-20	100.0 (153/153)	100.0 (153/153)	100.0 (60/60)	100.0 (366/366)	9.6	8.7-10.8
*Haemophilus influenzae**	1-24	100.0 (162/162)	100.0 (162/162)	100.0 (60/60)	100.0 (384/384)	13.6	11.0-21.6
Pseudomonas aeruginosa	5-17	100.0 (162/162)	100.0 (162/162)	100.0 (60/60)	100.0 (384/384)	14.7	12.8-17.6
Staphylococcus aureus	5-16	100.0 (171/171)	100.0 (171/171)	100.0 (60/60)	100.0 (402/402)	12.7	11.5-15.0
Streptococcus pneumoniae	1-29	100.0 (162/162)	100.0 (162/162)	100.0 (60/60)	100.0 (384/384)	12.0	9.6-15.6

* Tested with 4 mL blood.

BACT/ALERT® 3D and BACT/ALERT® VIRTUO® Systems Comparative Data

Analytical Sensitivity: Growth Performance
Data in the following table represent results from in-house seeded studies with blood (4 mL) obtained from healthy human donors. A single strain was tested for each species at target inoculum levels of ≤30 CFU/bottle. The actual inoculum levels ranged from 7-30 CFU/bottle for both BACT/ALERT® 3D and BACT/ALERT® VIRTUO®. Two positive bottles were subcultured per organism to assess purity. The species listed are representatives of clinically prevalent organisms in blood cultures.

Table 14: Analytical Sensitivity: Growth Performance

Microorganism	BACT/ALERT® PF Plus BACT/ALERT® VIRTUO® - 4 mL Blood				BACT/ALERT® PF Plus BACT/ALERT® 3D - 4 mL Blood			
	% Recovery (n)	Average CFU/ Bottle	Time to Detection		% Recovery (n)	Average CFU/ Bottle	Time to Detection	
			Mean	Range			Mean	Range
Abiotrophia defectiva	100.0 (9/9)	23	14.8	14.2-16.1	100.0 (4/4)	15	23.1	19.0-28.6
Aggregatibacter actinomycetemcomitans	100.0 (9/9)	21	29.8	27.2-32.2	100.0 (9/9)	21	32.6	28.1-34.8
Campylobacter jejuni	100.0 (9/9)	7	38.2	34.2-41.5	100.0 (9/9)	7	40.6	37.7-43.2
Candida albicans	100.0 (9/9)	11	26.9	23.4-29.8	100.0 (9/9)	11	28.3	26.6-31.0
Candida glabrata	100.0 (9/9)	7	41.7	38.4-44.5	100.0 (9/9)	7	49.4	45.6-55.9
Candida krusei	100.0 (9/9)	20	16.8	16.1-17.8	100.0 (9/9)	20	19.1	18.7-19.7
Cardiobacterium hominis	100.0 (9/9)	12	41.6	39.7-44.5	100.0 (9/9)	12	52.9	50.9-55.0
Corynebacterium jeikeium	100.0 (9/9)	8	39.5	29.9-50.7	100.0 (9/9)	8	84.4	52.1-112.8
Cryptococcus neoformans	100.0 (7/7)	30	56.6	49.5-65.8	100.0 (9/9)	30	57.8	56.4-59.0
Eikenella corrodens	100.0 (9/9)	24	21.2	20.1-22.5	100.0 (9/9)	24	24.7	24.0-25.4
Enterobacter aerogenes	100.0 (9/9)	10	10.7	10.3-11.1	100.0 (9/9)	10	12.4	12.0-12.7
Enterococcus faecalis	100.0 (9/9)	11	10.1	9.4-10.8	100.0 (9/9)	11	12.2	12.0-12.7
Escherichia coli	100.0 (9/9)	9	9.0	8.6-9.6	100.0 (9/9)	9	10.9	9.1-11.3
Haemophilus influenzae	100.0 (9/9)	18	14.8	13.5-16.3	100.0 (9/9)	18	17.8	17.0-18.5
Klebsiella pneumoniae	100.0 (9/9)	9	9.3	8.8-9.9	100.0 (9/9)	9	11.8	11.8-12.0
Listeria monocytogenes	100.0 (9/9)	19	18.9	18.2-20.0	100.0 (9/9)	19	20.5	20.2-20.9
Micrococcus luteus	100.0 (9/9)	24	31.2	30.0-32.4	100.0 (9/9)	24	33.2	32.4-33.8
Neisseria meningitidis	100.0 (9/9)	8	22.2	21.1-23.8	100.0 (9/9)	8	23.8	22.1-25.0
Proteus vulgaris	100.0 (9/9)	22	11.4	11.0-12.1	100.0 (9/9)	22	13.4	13.2-13.4
Pseudomonas aeruginosa	100.0 (9/9)	12	14.1	13.8-14.6	100.0 (9/9)	12	16.8	16.6-17.0
Salmonella enterica	100.0 (9/9)	9	11.0	10.6-11.4	100.0 (9/9)	9	13.1	12.5-13.9
Serratia marcescens	100.0 (9/9)	10	11.2	10.7-12.2	100.0 (9/9)	10	13.0	12.5-13.2
Staphylococcus aureus	100.0 (9/9)	14	10.4	10.1-10.8	100.0 (9/9)	15	13.3	12.7-13.9
Staphylococcus epidermidis	100.0 (9/9)	11	14.6	14.1-15.8	100.0 (9/9)	11	17.2	16.6-17.8
Stenotrophomonas maltophilia	100.0 (7/7)	22	65.1	24.8-83.9	100.0 (9/9)	22	33.5	31.4-37.0
Streptococcus agalactiae	100.0 (9/9)	13	11.0	10.5-12.2	100.0 (9/9)	13	14.3	13.9-14.6
Streptococcus mitis	100.0 (9/9)	17	8.8	8.6-9.0	100.0 (9/9)	17	11.9	11.5-12.0
Streptococcus pneumoniae	100.0 (9/9)	22	11.2	10.6-12.1	100.0 (9/9)	22	13.8	13.4-14.2
Streptococcus pyogenes	100.0 (9/9)	16	10.4	9.8-10.9	100.0 (9/9)	16	12.7	12.5-13.0

Clinical Study Results (Blood Cultures)
Results compare BACT/ALERT® VIRTUO® to BACT/ALERT® 3D with BACT/ALERT® PF Plus bottles for blood cultures (for all compliant pairs).

A multi-center clinical study was conducted at three different geographic sites in the U.S. comparing the performance of the BACT/ALERT® VIRTUO® and BACT/ALERT® 3D for aerobic culture pairs in which each bottle was filled with up to 4 mL of blood and [1) if the total volume of blood collected was >5 mL, the blood volume of the bottle with the smallest volume was within 20% of that of the bottle with the largest volume or 2) if the total volume of blood collected was ≤5 mL, the blood volume of the bottle with the smallest volume was within 50% of that of the bottle with the largest volume] (compliant pairs). A total of 161 bottle pairs were obtained from 62 pediatric patients suspected of blood stream bacterial/yeast infections. Subcultures of both bottles were performed when either bottle in the set was determined to be positive by the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D System. A pair of bottles was determined to have a positive status if the subculture of either the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D PF Plus culture bottle was positive. A culture bottle was determined to be a "True Positive" if the culture was flagged positive by the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D System and resulted in growth of the isolate upon subculture of this bottle. True positive rates were calculated for the BACT/ALERT® VIRTUO® PF Plus and BACT/ALERT® 3D PF Plus culture bottles, and the ratio of BACT/ALERT® VIRTUO® PF Plus true positives to BACT/ALERT® 3D PF Plus true positives was calculated to compare performance. Clinical isolates recovered were classified as significant, contaminant, or unknown based on determination by the clinical trial sites.

A total of 2 isolates were recovered from all compliant aerobic blood culture pairs with a positive status. There were a total of 2 bottle pairs that recovered a single isolate by subculture of BACT/ALERT® VIRTUO® or BACT/ALERT® 3D PF Plus culture bottles. The total population reported in the following table comprises the 2 isolates recovered from positive bottle pairs and 159 negative bottle pairs for a total of 161 results. The BACT/ALERT® VIRTUO® PF Plus culture bottle detected a total of 1 isolate compared to the BACT/ALERT® 3D PF Plus culture bottle that detected 1 isolate. Of the significant isolates, the BACT/ALERT® VIRTUO® PF Plus culture bottle detected a total of 1 isolate compared to the BACT/ALERT® 3D PF Plus culture bottle that detected 1 isolate. One false positive was identified by subculture of positive BACT/ALERT® VIRTUO® PF Plus culture bottles in the study population 0.62% (1/161). No false positives were identified by subculture of positive BACT/ALERT® 3D PF Plus culture bottles in the study population (0/161).

The following table compares results of the BACT/ALERT® VIRTUO® to BACT/ALERT® 3D blood cultures for all compliant BACT/ALERT® PF Plus blood culture bottles that yielded a single isolate on subculture (Table 15). No compliant aerobic blood culture pairs with a positive status yielded multiple isolates on subculture.

Table 15: Compliant – Single Isolates

Clinical Determination	BACT/ALERT® VIRTUO® True Positives	% of BACT/ALERT® VIRTUO® True Positives in Population	BACT/ALERT® 3D True Positives	% of BACT/ALERT® 3D True Positives in Population	Ratio of True Positives*	95% CI (LCL, UCL)
Significant	1	0.6 (1/161)	1	0.6 (1/161)	1.000	-1.772, 3.772*
Contaminant	0	0.0 (0/161)	0	0.0 (0/161)	-	-
Unknown	0	0.0 (0/161)	0	0.0 (0/161)	-	-
Total	1	0.6 (1/161)	1	0.6 (1/161)	1.000	-1.772, 3.772*

*Since the confidence interval contains a negative value and the ratio cannot be negative, the interval does not provide a meaningful interpretation.

A comparative yield of microorganisms (number of isolates) from BACT/ALERT® VIRTUO® and BACT/ALERT® 3D recovered on subculture of BACT/ALERT® PF Plus culture bottles is presented in the following table.

Table 16: Comparative Yield of Microorganisms (Number of Isolates) – Blood Cultures

Group	BACT/ALERT® VIRTUO®	BACT/ALERT® 3D
Enterobacteriaceae	1	0
Enterococcus spp.	-	-
Yeasts	-	-
Non-fermentative Gram-Negative Bacilli	-	-
Other Gram-Positive	-	-

Group	BACT/ALERT® VIRTUO®	BACT/ALERT® 3D
Coagulase-Negative *Staphylococcus*	-	-
Staphylococcus aureus	0	1
Streptococcus spp.	-	-
Other	-	-

In this clinical study, there were 196 pairs of BACT/ALERT® VIRTUO® and BACT/ALERT® 3D culture bottles with negative instrument results for both systems after 5 days of incubation. Among these pairs, terminal subcultures were performed for 50 pairs, and no false negative result by either BACT/ALERT® VIRTUO® or BACT/ALERT® 3D was observed; subculture on BACT/ALERT® 3D bottles alone was performed for 1 pair, and no false negative result was observed; both subcultures were not performed for 145 pairs of bottles. A culture bottle was determined to be false negative if the bottle result was negative by the instrument and resulted in growth upon subculture of the bottle.

Table 17: Summary of Percent False Negatives from Aerobic Blood Culture Pairs That Were Flagged Negative by Both Instruments

Subculture Performed BACT/ALERT® VIRTUO®	Subculture Performed BACT/ALERT® 3D	% False Negative BACT/ALERT® VIRTUO®	% False Negative BACT/ALERT® 3D
Yes	Yes	0.0 (0/50)	0.0 (0/50)
No	Yes	-	0.0 (0/1)

Clinical Study Results (Blood Cultures) - Low Fill BACT/ALERT® FA Plus Culture Bottles
Results compare BACT/ALERT® VIRTUO® to BACT/ALERT® 3D with low fill BACT/ALERT® FA Plus bottles for blood cultures (for all compliant pairs).

Due to the low numbers of positive BACT/ALERT® PF Plus bottles observed in the clinical study, low fill volume BACT/ALERT® FA Plus bottles were evaluated as a surrogate for BACT/ALERT® PF Plus as both are equal in terms of the medium composition. Note that the bottle labeling for BACT/ALERT® PF Plus and BACT/ALERT® FA Plus bottles provide the recommended blood volumes for each bottle type and differentiate their respective use in a clinical setting. A multi-center clinical study was conducted at three different geographic sites in the U.S. and Canada comparing the performance of the BACT/ALERT® VIRTUO® and BACT/ALERT® 3D for aerobic culture pairs in which each bottle was filled with up to 4 mL of blood and in which the blood volume of the bottle with the smallest volume was within 30% of that of the bottle with the largest volume (compliant pairs). A total of 379 bottle pairs were obtained from 292 adult patients suspected of blood stream bacterial/yeast infections. Subcultures of both bottles were performed when either bottle in the set was determined to be positive by the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D System. A pair of bottles was determined to have a positive status if the subculture of either the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D PF Plus culture bottle was positive. A culture bottle was determined to be a "True Positive" if the culture was flagged positive by the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D System and resulted in growth of the isolate upon subculture of this bottle. True positive rates were calculated for the BACT/ALERT® VIRTUO® FA Plus and BACT/ALERT® 3D FA Plus culture bottles, and the ratio of BACT/ALERT® VIRTUO® FA Plus true positives to BACT/ALERT® 3D FA Plus true positives was calculated to compare performance. Clinical isolates recovered were classified as significant, contaminant, or unknown based on determination by the clinical trial sites.

A total of 40 isolates were recovered from all compliant aerobic blood culture pairs with a positive status. There were a total of 40 bottle pairs that recovered a single isolate by subculture of BACT/ALERT® VIRTUO® or BACT/ALERT® 3D FA Plus culture bottles. The total population reported in Table 18 comprises the 40 isolates recovered from positive bottle pairs and 339 negative bottle pairs for a total of 379 results. The BACT/ALERT® VIRTUO® FA Plus culture bottle detected a total of 33 isolates compared to the BACT/ALERT® 3D FA Plus culture bottle that detected 32 isolates. Of the significant isolates, the BACT/ALERT® VIRTUO® FA Plus culture bottle detected a total of 28 isolates compared to the BACT/ALERT® 3D FA Plus culture bottle that detected 29 isolates. No false positives were identified by subculture of positive BACT/ALERT® VIRTUO® FA Plus culture bottles in the study population (0/379). No false positives were identified by subculture of positive BACT/ALERT® 3D FA Plus culture bottles in the study population (0/379).

The following table compares results of the BACT/ALERT® VIRTUO® to BACT/ALERT® 3D blood cultures for all compliant low fill BACT/ALERT® FA Plus blood culture bottles that yielded a single isolate on subculture (Table 18). No compliant aerobic blood culture pairs with a positive status yielded multiple isolates on subculture.

Table 18: Low Fill BACT/ALERT® FA Plus – Blood – Compliant – Single Isolates

Clinical Determination	BACT/ALERT® VIRTUO® True Positives	% of BACT/ALERT® VIRTUO® True Positives in Population	BACT/ALERT® 3D True Positives	% of BACT/ALERT® 3D True Positives in Population	Ratio of True Positives	95% CI (LCL, UCL)
Significant	28	7.4 (28/379)	29	7.7 (29/379)	0.966	0.790, 1.142
Contaminant	4	1.1 (4/379)	3	0.8 (3/379)	1.333	-
Unknown	1	0.3 (1/379)	0	0.0 (0/379)	-	-
Total	33	8.7 (33/379)	32	8.4 (32/379)	1.031	0.790, 1.272

A comparative yield of microorganisms (number of isolates) from BACT/ALERT® VIRTUO® and BACT/ALERT® 3D recovered on subculture of compliant low fill BACT/ALERT® FA Plus culture bottles is presented in the following table.

Table 19: Comparative Yield Of Microorganisms (Number of Isolates) – Low Fill BACT/ALERT® FA Plus Blood Cultures

Group	BACT/ALERT® VIRTUO®	BACT/ALERT® 3D
Enterobacteriaceae	8	9
Enterococcus spp.	3	2
Yeasts	2	1
Non-fermentative Gram-Negative Bacilli	2	1
Other Gram-Positive	0	1
Coagulase-Negative *Staphylococcus*	5	5
Staphylococcus aureus	6	7
Streptococcus spp.	6	5
Other	1	1

In this clinical study, there were 492 pairs of BACT/ALERT®VIRTUO® and BACT/ALERT® 3D culture bottles with negative instrument results for both systems after 5 days of incubation. Among these pairs, terminal subcultures were performed for 242 pairs, and no false negative result by either BACT/ALERT® VIRTUO® or BACT/ALERT® 3D was observed; subculture on BACT/ALERT® VIRTUO® bottles alone was performed for 4 pairs, and no false negative result was observed; subculture on BACT/ALERT® 3D bottles alone was performed for 3 pairs, and no false negative result was observed; both subcultures were not performed for 243 pairs of bottles. A culture bottle was determined to be false negative if the bottle result was negative by the instrument and resulted in growth upon subculture of the bottle.

Table 20: Summary of Percent False Negatives From Low Fill BACT/ALERT® FA Plus Aerobic Blood Culture Pairs That Were Flagged Negative By Both Instruments

Subculture Performed BACT/ALERT® VIRTUO®	Subculture Performed BACT/ALERT® 3D	% False Negative BACT/ALERT® VIRTUO®	% False Negative BACT/ALERT® 3D
Yes	Yes	0.0 (0/242)	0.0 (0/242)
Yes	No	0.0 (0/4)	-
No	Yes	-	0.0 (0/3)

Summary of False Positive Results

A culture bottle was determined to be a false positive if the culture was flagged positive by the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D System and was negative upon subculture of the bottle. The study population consisted of culture pairs that received blood volumes of ≤4 mL (BACT/ALERT® PF Plus and low fill BACT/ALERT® FA Plus bottles). The false positive results were identified by subculture of positive BACT/ALERT® VIRTUO® bottles and positive BACT/ALERT® 3D bottles, comprising proportions of the study populations based on the total numbers of corresponding blood cultures.

Table 21: Summary of False Positive Results

Bottle Type - Specimen Type	% False Positive BACT/ALERT® VIRTUO®	% False Positive BACT/ALERT® 3D
BACT/ALERT® PF Plus - Blood	0.50 (1/202)	0.0 (0/202)
Low Fill BACT/ALERT® FA Plus - Blood	0.0 (0/550)	0.0 (0/550)

Limited Warranty

bioMérieux warrants the performance of the product for its stated intended use provided that all procedures for usage, storage and handling, shelf life (when applicable), and precautions are strictly followed as detailed in the instructions for use (IFU).

Except as expressly set forth above, bioMérieux hereby disclaims all warranties, including any implied warranties of merchantability and fitness for a particular purpose or use, and disclaims all liability, whether direct, indirect or consequential, for any use of the reagent, software, instrument and disposables (the "System") other than as set forth in the IFU.

Availability

BIOMÉRIEUX		
BACT/ALERT® PF Plus	100/case	REF 410853

For technical assistance in the USA, contact bioMérieux Customer Service at 1-800-682-2666. Outside the USA, contact your local bioMérieux representative.

Index of Symbols

Symbol	Meaning
	Catalogue number
	Manufacturer
	Date of manufacture
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Authorized Representative in the European Community
	This way up

Symbol	Meaning
IVD	*In Vitro* Diagnostic Medical Device
	Do not reuse
LATEX	Does not contain latex
Rx only	For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner

Instructions for use provided in the kit or downloadable from www.biomerieux.com/techlib

Revision History

Change type categories

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release Date	Part Number	Change Type	Change Summary
2020-07	056200-01	Technical change	**Reagents** - Expanded description of potential formulation adjustments.
			Specimen Collection and Preparation - Direct Draw Procedure - Added step regarding proper mixing after inoculation
			BACT/ALERT PF Plus Culture Bottle Test Procedure - Added precaution regarding personal protective equipment (PPE)
			Neutralization of Antimicrobials and **Performance Characteristics** - Added information regarding equivalency testing after formulation adjustment
2017-04	9313400 E	Technical change	Addition of VIRTUO® information throughout, including **Expected Values**, **Neutralization of Antimicrobials**, and **Performance Characteristics of the Test** (Tables 11-21) sections.

Release Date	Part Number	Change Type	Change Summary
2016-04	9309505 D	Technical change	**Reagents** • Update to composition information • Clarification of expiration date
			Specimen Collection and Preparation - Addition of Caution regarding bottle pressure
			Quality Control - Addition of Caution regarding LIS and bottle type abbreviations
			Addition of Rx - only caution and symbol for US customers
		Administrative	**Limited Warranty** - Addition of statement
			Index of Symbols - Update to reflect new symbols on product
2013-04	9305130 C	Technical Change	**Intended Use, Specimen Collection and Preparation, Expected Values, Neutralization of Antimicrobials, Performance Characteristics of the Test** - Revised text to include additional information on product performance based on clinical studies
			Limitations of the Test - Added Limitations 2, 8, and 9 following FDA review
		Administrative	**Reagents**: Moved Cautions to Limitations of the Test section

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