

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k121446

B. Purpose for Submission:

To obtain a substantial equivalent determination for a premarket notification for the BacT/ALERT[®] PF Plus Culture Bottle

C. Measurand:

Aerobic and anaerobic facultative microorganisms (bacteria and yeast)

D. Type of Test:

Liquid culture medium for recovery of microorganisms (bacteria and yeast) from blood using colorimetric sensor to detect CO₂ dissolved in the culture media.

E. Applicant:

bioMérieux, Inc

F. Proprietary and Established Names:

BacT/ALERT[®] PF Plus Culture Bottles

G. Regulatory Information:

1. Regulation section:

21 CFR 866.2560, Microbial Growth Monitor

2. Classification:

Class I

3. Product code:

MDB

4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use:

BacT/ALERT® PF Plus Culture Bottles are used with the BacT/ALERT® Microbial Detection System in qualitative procedures for enhanced recovery and detection of aerobic and facultative anaerobic microorganisms (bacteria and yeast) from blood.

2. Indications for use:

BacT/ALERT® PF Plus Culture Bottles are used with the BacT/ALERT® Microbial Detection System in qualitative procedures for enhanced recovery and detection of aerobic and facultative anaerobic microorganisms (bacteria and yeast) from blood.

3. Special conditions for use statement(s):

Prescription use

4. Special instrument requirements:

BacT/ALERT® Microbial Detection System

I. Device Description:

The BacT/ALERT Microbial Detection System provides both a microbial detection system and a culture medium bottle with suitable nutritional and environmental conditions for microorganisms commonly encountered in blood taken from a patient suspected of having bacteremia/fungemia. In the BacT/ALERT PF Plus bottle, charcoal is eliminated and is replaced with two types of absorbent resins as the antimicrobial neutralization agents. The PF Plus bottle is to increase the clarity of Gram stains in comparison to the predicate BacT/ALERT PF Culture Bottle.

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 bottles.

The BacT/ALERT Microbial Detection System utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO₂) that is dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide is produced as the microorganisms metabolize the substrates in the

culture medium. When growth of the microorganisms produces CO₂, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow. 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.

J. Substantial Equivalence Information:

1. Predicate device name(s):

BacT/ALERT PF Culture Bottle

2. Predicate K number(s):

k020923

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Qualitative procedure for the aerobic culture and recovery of microorganisms (bacteria and yeast) from blood, with the BacT/ALERT Microbial Detection System	Same
Specimen type	Human blood	Same
Instrumentation	BacT/ALERT Microbial Detection System	Same
Detection Technology	Continuous monitoring; utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO ₂) that is dissolved in the culture medium, produced by the growth of aerobic and facultative anaerobic bacteria and yeast	Same

Differences		
Item	Device	Predicate
Medium Fill Volume	30 mL	20 mL
Antimicrobial neutralization properties	Dry adsorbent polymeric beads	Charcoal Slurry
Media formulation	Casein Peptone, 1.0% w/v	Soybean-casein Digest, 2.0% w/v
	Yeast Extract, 0.45% w/v	Brain Heart Infusion Solids, 0.1% w/v
	Sodium Polyanethol Sulfonate (SPS), 0.083% w/v	SPS, 0.025% w/v
	Pyridoxine HCl, 0.001% w/v	Same
	Menadione, 0.00005% w/v	Menadione, 0.0000625% w/v
	Hemin, 0.0005% w/v	Hemin, 0.000625% w/v
	L-cysteine, 0.03% w/v	L-cysteine, 0.025% w/v
	Soybean Peptone, 0.3% w/v Meat Peptone, 0.1% w/v Pyruvic Acid, 0.1% w/v Nicotinic Acid, 0.0002% w/v Pantothenic Acid, 0.0002% w/v Thiamine HCl, 0.0001% w/v	None
Headspace Gases	O ₂ , CO ₂ , N ₂ under vacuum	Different composition of O ₂ , CO ₂ , N ₂ under vacuum
Blood sample volume	0.1 to 4.0 mL	Up to 4.0 mL

K. Standard/Guidance Document Referenced (if applicable):

Clinical and Laboratory Standards Institute (CLSI) M22-A3, Quality Control for Commercially Prepared Microbiology Culture Media, 2004

CLSI M47-A, Principles and Procedures for Blood Cultures; Approved Guidelines, 2007

CLSI M100-S21, Performance standards for antimicrobial susceptibility testing, twenty-first informational supplement, 2011

CLSI EP5-A2, Evaluation of Precision Performance of Clinical Chemistry Devices, Approved Guidelines, 2004

CLSI EP 12-A2, User Protocol for Evaluation of Qualitative Test Performance, Approved Guideline, 2008

L. Test Principle:

The BacT/ALERT (BTA) Microbial Detection System utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO₂) that is dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide is produced as the microorganisms metabolize the substrates in the culture medium. When growth of the microorganisms produces CO₂, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow. 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

Antimicrobial Neutralization

An internal seeded study was conducted to demonstrate an acceptable rate of recovery and detection of microorganisms in the presence of antimicrobials. The targeted inoculum for each organism was 125 CFU/bottle. Bottles without adsorbent (i.e. SA) were used as negative controls during the study. In this seeded study, blood containing antimicrobials, in clinically relevant concentrations, was added directly to the BacT/ALERT[®] PF Plus Culture Bottles and inoculated with susceptible strains of aerobic and facultative anaerobic microorganisms. Antimicrobial neutralization was tested on at least three validation lots of PF Plus Culture Bottles. Initial testing of all antimicrobials was evaluated at 120% Peak Serum Level (PSL). When bottles fail to recover microorganism for a particular antimicrobial/microorganism combination when the antimicrobial is at 120% PSL, that antimicrobial/microorganism pair was tested at 100% PSL or lower as necessary to achieve 100% recovery of the organism seeded. Antimicrobials were considered effectively neutralized by the BacT/ALERT PF Plus medium based on 100% recovery of the organisms tested at the indicated percent of peak serum level tested.

The study demonstrated that antimicrobials from the following categories were neutralized by BacT/ALERT[®] PF Plus Culture Bottle resulting in a positive bottle signal within five days or less after inoculation: penicillins, glycolcyclines, polyenes, macrolides, triazoles, echinocandins, aminoglycosides, fluoroquinolones, lincosamides, glycopeptides, and oxazolidinones. Antimicrobial neutralization was also achieved for cefazolin, cefoxitin, ceftaroline.

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% PSL to 2% PSL depending on the microorganism. Ceftriaxone was neutralized at ranges of 50% PSL to 1% PSL depending on the microorganism. The antimicrobial neutralization study results

are summarized in Table 1 below.

Table 1 Antimicrobial Neutralization Study Results.

Antimicrobial	PSL concentration (µg/ml)*	Antimicrobial Class	Organism	MIC (µg/ml) ^{1,2,3,4,5,6,7}	Initial testing concentration (µg/ml)*	% of PSL	% Recovery (n/N)**
Amikacin	30	Aminoglycoside	<i>Pseudomonas aeruginosa</i>	1 - 4	7.5	100%	100% (17/17)
	36	Aminoglycoside	<i>Escherichia coli</i>	0.5 - 4	9	120%	100% (17/17)
Amphotericin B	4.2	Polyene	<i>Candida albicans</i>	0.25 - 1	1.1	120%	100% (17/17)
Ampicillin	47	b-lactam	<i>Enterococcus faecalis</i>	0.5 - 2	11.8	100%	100% (17/17)
			<i>Escherichia coli</i>	2 - 8	11.8	100%	100% (17/17)
Ampicillin + sulbactam	47 / 28	b-lactam, b-lactamase inhibitor combination	<i>Escherichia coli</i>	2/1 - 8/4	11.8 / 7	50%	100% (17/17)
Azithromycin	4.3	Macrolide	<i>Streptococcus pneumoniae</i>	0.06 - 0.25	1.1	120%	100% (17/17)
Caspofungin	11.9	Echinocandin	<i>Candida albicans</i>	0.1	3	120%	100% (17/17)
Cefazolin	8	1st generation Cephalosporin	<i>Staphylococcus aureus</i>	0.25 - 1	2	4%	100% (17/17)
	94	1st generation Cephalosporin	<i>Escherichia coli</i>	1 - 4	23.5	50%	100% (17/17)
Cefotaxime	2	3rd generation Cephalosporin	<i>Escherichia coli</i>	2 - 8	0.5	2%	100% (17/17)
	50	3rd generation Cephalosporin	<i>Staphylococcus aureus</i>	1 - 4	12.5	50%	100% (17/17)
Cefoxitin	110	2nd generation Cephalosporin	<i>Escherichia coli</i>	1 - 4	27.5	100%	100% (17/17)
	110	2nd generation Cephalosporin	<i>Staphylococcus aureus</i>	1 - 4	27.5	100%	100% (17/17)
Ceftaroline	21	4th generation Cephalosporin	<i>Escherichia coli</i>	0.03 - 0.12	5.3	100%	100% (17/17)
	21	4th generation Cephalosporin	<i>Staphylococcus aureus</i>	0.12 - 0.5	5.3	100%	100% (17/17)
Ceftriaxone	0.96	3rd generation Cephalosporin	<i>Escherichia coli</i>	0.03 - 0.12	0.24	1%	100% (17/17)
	75	3rd generation Cephalosporin	<i>Staphylococcus aureus</i>	1 - 8	18.8	50%	82.4% (14/17)
Ciprofloxacin	5.5	Fluoroquinolone	<i>Pseudomonas aeruginosa</i>	0.25 - 1	1.4	120%	100% (17/17)
Clarithromycin	4.8	Macrolide	<i>Staphylococcus aureus</i>	0.12 - 0.5	1.2	120%	100% (17/17)
Clindamycin	12	Lincosamide	<i>Staphylococcus aureus</i>	0.06 - 0.25	3	120%	100% (17/17)

Antimicrobial	PSL concentration (µg/ml)*	Antimicrobial Class	Organism	MIC (µg/ml) ^{1,2,3,4,5,6,7}	Initial testing concentration (µg/ml)*	% of PSL	% Recovery (n/N)**
Daptomycin	119	Lipopeptide	<i>Staphylococcus aureus</i>	0.12 - 1	29.8	120%	100% (17/17)
Erythromycin Oral	2.4	Macrolide	<i>Staphylococcus aureus</i>	0.25 - 1	0.6	120%	100% (17/17)
Fluconazole	16.8	Azole	<i>Candida albicans</i>	0.25 - 0.5	4.2	120%	100% (17/17)
Gentamicin	12	Aminoglycoside	<i>Escherichia coli</i>	0.25 - 1	3	120%	100% (17/17)
Levofloxacin	8.6	Fluoroquinolone	<i>Staphylococcus aureus</i>	0.06 - 0.5	2.2	100%	100% (17/17)
	10.3	Fluoroquinolone	<i>Enterococcus faecalis</i>	0.25 - 2	2.6	120%	100% (17/17)
Linezolid	24	Oxazolidinone	<i>Enterococcus faecalis</i>	1 - 4	6	120%	100% (17/17)
Micafungin	19.2	Echinocandin	<i>Candida albicans</i>	0.015	4.8	120%	100% (17/17)
Moxifloxacin	4.5	Fluoroquinolone	<i>Escherichia coli</i>	0.008 - 0.06	1.1	100%	100% (17/17)
Ofloxacin	4.6	Fluoroquinolone	<i>Staphylococcus aureus</i>	0.12 - 1	1.2	100%	100% (17/17)
Oxacillin	18	b-lactam	<i>Staphylococcus aureus</i>	0.12 - 0.5	4.5	120%	100% (17/17)
Penicillin G	24	b-lactam	<i>Streptococcus pneumoniae</i>	0.25 - 1	6	120%	100% (17/17) (w/ blood)
Piperacillin	480	b-lactam	<i>Pseudomonas aeruginosa</i>	1 - 8	120	120%	100% (17/17)
Piperacillin + Tazobactam	190 / 19	b-lactam, b-lactamase inhibitor combination	<i>Pseudomonas aeruginosa</i>	1/4 - 8/4	47.5 / 4.7	100%	100% (17/17)
	228 / 22.8	b-lactam, b-lactamase inhibitor combination	<i>Staphylococcus aureus</i>	0.25/4 - 2/4	57 / 5.7	120%	100% (18/18)
Telithromycin	2.3	Ketolide	<i>Staphylococcus aureus</i>	0.06 - 0.25	0.58	100%	100% (17/17)
Tigecycline	0.63	Glycylcycline	<i>Escherichia coli</i>	0.03 - 0.25	0.16	100%	100% (17/17)
			<i>Streptococcus pneumoniae</i>	0.015 - 0.12	0.16	100%	100% (17/17)
	0.76	Glycylcycline	<i>Staphylococcus aureus</i>	0.03 - 0.25	0.19	120%	100% (17/17)
Tobramycin	12	Ketolide	<i>Escherichia coli</i>	0.25 - 1	3	120%	100% (17/17)
Trimethoprim / sulfamethoxazole	11 / 126	Sulfonamide combination	<i>Escherichia coli</i>	<= 0.5/9.5	2.7 / 31.5	120%	100% (17/17)

Antimicrobial	PSL concentration (µg/ml)*	Antimicrobial Class	Organism	MIC (µg/ml) ^{1,2,3,4,5,6,7}	Initial testing concentration (µg/ml)*	% of PSL	% Recovery (n/N)**
			<i>Streptococcus pneumoniae</i>	0.12/2.4 - 1/19	2.7 / 31.5	100%	100% (17/17)
Vancomycin	60	Glycopeptide	<i>Staphylococcus aureus</i>	0.5 - 2	15	120%	100% (17/17)
Voriconazole	3.6	Azole	<i>Candida albicans</i>	N/A	0.9	120%	100% (19/19)
			<i>Candida parapsilosis</i>	0.03 - 0.25	0.9	120%	100% (17/17)

* Antimicrobial Therapy Inc. 2009 Sanford guide to antimicrobial therapy. Sperryville, VA.

** Growth of susceptible microorganism in the presence of antimicrobial

References Cited in the above table:

1. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S22. Wayne, Pa; Clinical and Laboratory Standards Institute; 2012.
2. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Twenty-Seventh Informational Supplement. CLSI document M27-S1. Wayne, Pa; Clinical and Laboratory Standards Institute; in press.
3. Li XC, MR Jacob, SI Khan, MK Ashfaq, S Babu, AK Agarwal, HN El Sohly, SP Manly, and AM Clark. 2008. Potential In Vitro Antifungal Activities of Naturally Occurring Acetylenic Acids. *Antimicrobial Agents and Chemotherapy*. 52: 2442-2448.
4. Pai, Manjunath P. 2009. Antifungal Combinations against Simulated *Candida albicans* Endocardial Vegetations. *Antimicrobial Agents and Chemotherapy*. 53: 2626-2631.
5. Espinel-Ingroff A., JL Rodrigues-Tudela, and JV Martinez-Suarez. 1995. Comparison of two alternative microdilution procedures with the National Committee for Clinical Laboratory Standards reference microdilution method M27-P for in vitro testing of fluconazole-resistant and -susceptible isolates of *Candida albicans*. *Journal of Clinical Microbiology* 33: 3145-3158.
6. Klepser ME, EJ Wolfe, RN Jones, CH Nightingale, and MA Pfaller. 1997. Antifungal pharmacodynamic characteristics of fluconazole and amphotericin B tested against *Candida albicans*. *Antimicrobial Agents and Chemotherapy*. 41: -1392-1395.
7. Marchetti O, P Moreillon, MP Glauser, J Bille, and D Sanglard. 2000. Potent Synergism of the Combination of Fluconazole and Cyclosporine in *Candida albicans*. *Antimicrobial Agents and Chemotherapy*. 44: 2373-2381

Delayed Entry

A multi-center study was conducted to evaluate the effect of a delay, from the time the BacT/ALERT[®] PF Plus is inoculated, to the time the bottle is placed on the instrument. This seeded study was conducted at three study sites (one in-house, and two external sites), each using a different lot of BacT/ALERT[®] PF Plus Culture Bottles. Three replicates each of 11 species* at target concentrations 100 CFU per bottle (acceptable range of 30 to 300 CFU per bottle) were generated at three sites. Actual inoculum levels ranged from 35 CFU/bottle to 290 CFU/bottle. All bottles contained human blood from healthy volunteers and were held at specified temperatures and times prior to loading into the BacT/ALERT instrument. Percent recovery reflects positive flag by

the instrument and Gram stain/subculture consistent with the seeded isolation of target organisms.

BacT/ALERT[®] PF Plus were tested at five different combinations of holding temperature/holding period, as indicated below, prior to being loaded on to the instrument. This was done to evaluate the instrument's ability to detect the organisms within 5 days of incubation based on a panel of microorganisms that have been seeded into simulated blood cultures.

The study demonstrated that all holding conditions showed $\geq 90\%$ percent recovery except for culture bottles held at 35 to 37°C for 24 hours or longer before loading. At this holding temperature/period combination, the PF Plus may not detect microorganisms and should be subcultured. The study results are illustrated in Table 2 below.

**Staphylococcus aureus, Candida albicans, Candida krusei, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Streptococcus pneumoniae, Enterococcus faecium, Haemophilus influenzae, Neisseria meningitidis*

Table 2. Delayed Entry Summary

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)**	-	-	N/A

** One (1) false positive result observed during seeded study. Negative confirmation by gram stain and subculture.

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

a. Precision/Reproducibility:

Within-Laboratory Precision (Repeatability)

Within-laboratory precision was evaluated 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 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 3. Within-Laboratory Precision (Repeatability) results

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

Reproducibility

A reproducibility study was conducted at three study sites (two external and one internal) using a target of at least 162 replicates per site on three (3) days with a minimum of two operators per site. Reproducibility was evaluated on each of 9 organisms. Two organisms (*C. albicans* and *S. pneumoniae*) were prepared by serial dilution and the other seven organisms were prepared using Bioballs. *C. albicans* and *S. pneumoniae* were seeded into the PF Plus bottle, at a target inoculum of 100 CFU/bottle, with an acceptable range of 30-300 CFU/bottle and the other 7 organisms at a target range of 1-17 CFU/bottle. The actual inoculum ranged from 6 CFU/bottle to 700 CFU/bottle for the 30-300 CFU/bottle range, and from 1 CFU/bottle to 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. The study results are illustrated in Table 4 below:

Table 4. Summary of Reproducibility Data

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

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

The above data includes 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 85.0% recovery for all sites combined.

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Stability of the BacT/ALERT PF Plus bottles (shelf life)

Studies were conducted to demonstrate the PF Plus bottles shelf-life. Three lots each of PF Plus bottles produced for product validation and for product verification were tested until they were 13 month old. At the beginning of the study, bottles were thermally stressed, or not thermally stressed, and then stored at either 15-30°C or 2-8°C for a total of 4 treatment/storage conditions. At each time point, growth performance, antimicrobial neutralization, and physical parameters were evaluated. Bottles were also tested to determine if they possessed sufficient vacuum to direct draw 10 ml blood, and if coagulation was prevented when direct blood draw was allowed to continue until bottle vacuum was exhausted. Initial stability studies were conducted using a high target inoculum and were not challenging enough based on the claimed LoD. Additional studies were conducted using levels of organisms around the LoD using aged bottles and demonstrated that stability can be achieved for the claimed shelf life.

It was noted that the vacuum remains as ≥ 11 Hg for the PF Plus. There are the potential risk(s) of drawing more than 4 mL of blood in the event of direct draw in the pediatric population, which could be as low as 50 mL. A caution statement was in place in the package insert.

Quality Control

Quality control testing was conducted at the clinical sites. Overall QC results were found to be acceptable at the clinical sites. Unacceptable QC results were observed due to technical errors such as colony counts out of range, site failure to change bottles status after positive instrument signal and positive subculture, and no supplement added.

The isolates listed below were inoculated with test organisms with growth within 5 days being the acceptable result. All test organisms were inoculated into PF Plus bottles at a target concentration of 100 CFUs.

Aerobic Quality Control Organisms

<i>Candida albicans</i>	ATCC 14053
<i>Candida krusei</i>	ATCC 14243
<i>Enterococcus faecalis</i>	ATCC 29212
<i>Escherichia coli</i>	ATCC 25922
<i>Haemophilus influenzae</i>	ATCC 10211
<i>Neisseria meningitidis</i>	ATCC 13090
<i>Pseudomonas aeruginosa</i>	ATCC 27853
<i>Staphylococcus aureus</i>	ATCC 25923
<i>Staphylococcus epidermidis</i>	ATCC 12228
<i>Stenotrophomonas maltophilia</i>	ATCC 13637
<i>Streptococcus agalactiae</i>	ATCC 13813

Streptococcus pneumoniae ATCC 6305
Streptococcus pyogenes ATCC 19615

d. *Detection limit:*

Limit of Detection (LoD)

A minimum of 30 replicates were tested on each organism in Table 5 below in an in-house seeded, using bottles at end of shelf life. All testing was done in the absence of blood with the exception of four mL pooled human blood for *Haemophilus influenzae*. At least 95% detection rate was achieved at LoD for the organisms tested below:

Table 5. Summary of LoD Data

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

NOTE: 96.7% of the bottles were subcultured within 30 minutes of being declared positive

Growth Performance

Data in Table 6 below represent results from in-house seeded study with blood obtained from healthy human volunteers. Multiple strains were tested for each species at target inoculum levels of 125 CFU per bottle. The actual inoculum levels ranged from 3 CFU/bottle to 298 CFU/bottle. In this seeded study BacT/ALERT PF Plus bottles were subcultured at least 24 hours after being flagged positive by the instrument. The microorganisms listed are representatives of clinically prevalent organisms in blood cultures.

Table 6. Summary of Growth Performance

Microorganism	Blood			
	% Recovery (n)	Range CFU/bottle	Time to Detection (hours)	
			Mean	Range
<i>Staphylococcus aureus</i>	100.0 (30/30)	54 - 150	13.3	12.2 - 15.2
<i>Escherichia coli</i>	100.0 (30/30)	71 - 254	11.2	10.3 - 11.7
<i>Pseudomonas aeruginosa</i>	100.0 (12/12)	74 - 148	15.7	13.7 - 17.8

<i>Klebsiella pneumoniae</i>	100.0 (12/12)	89 - 123	11.3	10.6 - 12.3
<i>Candida albicans</i>	100.0 (30/30)	88 - 298	29.0	19.2 - 52.8
<i>Streptococcus pneumoniae</i>	100.0 (30/30)	3 - 260	13.8	10.8 - 16.5
<i>Staphylococcus epidermidis</i>	100.0 (12/12)	44 - 135	17.6	14.3 - 18.8
<i>Enterococcus faecalis</i>	100.0 (12/12)	63 - 259	11.6	11.0 - 12.2
<i>Enterococcus faecium</i>	100.0 (12/12)	25 - 120	12.8	11.3 - 14.4
<i>Enterobacter cloacae</i>	100.0 (12/12)	111 - 200	11.6	10.8 - 12.5
<i>Candida glabrata</i>	100.0 (12/12)	118 - 281	43.5	27.3 - 64.8
<i>Haemophilus influenzae</i>	100.0 (12/12)	105 - 266	14.4	12.0 - 16.8
<i>Proteus mirabilis</i>	100.0 (12/12)	36 - 213	12.5	11.3 - 14.6

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

e. *Analytical specificity:*

Potentially Interfering Substances

A seeded study was conducted to demonstrate that the PF Plus bottle can detect microorganisms in the presence of interfering substances such as plasma, and SPS. The microorganisms were *S. aureus*, *N. meningitides*, and *H. influenzae*. No interferences observed.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Performance of the new BacT/ALERT[®] PF Plus Culture Bottles was compared to that of the BacT/ALERT[®] PF Culture Bottles.

Clinical Study Results (Blood Cultures)

A multi-center clinical study was conducted at three different geographic sites in the U.S. comparing the performance of the PF Plus and 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 PF Plus or PF

bottle was positive. A culture bottle was determined to be a “True Positive” if the bottle 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 PF Plus and PF culture bottles, and the ratio of PF Plus true positives to 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 bottle pairs with a positive status. There were a total of 145 bottle pairs that recovered at least 1 isolate by subculture of PF Plus or PF bottles. A total of 126 bottle pairs recovered a single isolate, 12 bottle pairs recovered two isolates, 6 bottle pairs recovered 3 isolates, and 1 bottle pair recovered 4 isolates. The total population reported in Table B 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 bottle detected a total of 140 isolates compared to the BacT/ALERT PF bottle that detected 128 isolates. Of the significant isolates, the BacT/ALERT PF Plus bottle detected a total of 91 isolates compared to the BacT/ALERT PF bottle that detected 77 isolates. One (1) false positive was identified by subculture of a positive BacT/ALERT PF Plus bottle and comprised 0.05% (1/2215) of the study population.

The tables below 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 (Tables A, B), a single isolate alone on subculture (Table C), and multiple isolates on subculture (Table D).

Table A. All compliant pairs with Single and Multiple Isolates

Positive Status	Result			Total
	PF Plus=TP PF=FN	PF Plus=FN PF=TP	PF Plus=TP PF=TP	
Significant	14	8	69	99
Contaminant	22	19	10	43
Unknown	8	5	17	30
Total	44	32	96	172

Table B. All Compliant Pairs with Single and Multiple Isolates Combined

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%	29	1.3%	0.828

		(24/2215)		(29/2215)	
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 PF Plus and PF, 44 isolates were detected only by PF Plus and 32 isolates were detected only by PF. The ratio of true positive rates for overall isolates was 1.094 with a 95% CI of (0.954, 1.234). For more details about study design and calculations of confidence intervals, see Kondratovich, M.V (2008) Comparing Two Medical Tests When Results of Reference Standard Are Unavailable for Those Negative via Both Tests, *Journal of Biopharmaceutical Statistics*, 18: 1; 145-166.

Table C. All Compliant Pairs with Single Isolates

Clinical Determination	PF Plus True Positives	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 PF Plus and PF, 32 isolates were detected only by PF Plus and 21 isolates were detected only by PF. The ratio of true positive rates for overall single isolates was 1.117 (105/94) with a 95% CI of (0.957, 1.277).

Table D. All Compliant Pairs with Multiple Isolates

Clinical Determination	PF Plus True Positives	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 PF Plus and PF, 12 isolates were detected only by PF Plus and 11 isolates were detected only by PF. The ratio of true positive rates for overall multiple isolates was 1.029 (35/34) with a 95% CI of (0.748, 1.310).

In this clinical study, there were 2041 pairs of PF Plus and PF bottles with negative instrument results for both bottles after 5 days of incubation. Among these pairs, terminal subcultures on both bottles were performed for 3 pairs, and 0 false negative results by both PF Plus and PF bottles were observed; subculture on PF Plus bottles alone was performed for 2034 pairs, and 1 false negative result was observed; both subcultures were not performed for 4 pairs of bottles. Results are summarized in Table E below.

Table E. Summary of Percent False Negatives from compliant pediatric blood culture pairs that were flagged negative by the instrument for both bottles

Subculture Performed PF Plus	Subculture Performed PF	% False Negative PF Plus	% False Negative PF
Yes	Yes	0.00% (0/3)	0.00% (0/3)
Yes	No	0.05% (1/2034)	NA

Overall false negative rate for 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 PF Plus and PF cultures are presented in Table F below:

Table F. Comparative Yield of Microorganisms Recovered

Organism Group	Pediatric Subgroup	BacT/ALERT PF Plus	BacT/ALERT PF Plus Fill Range (ml)	BacT/ALERT PF	BacT/ALERT PF Fill Range (ml)
<i>Enterobacteriaceae</i>	Newborn (< 1 mo)	6	0.1-1.4	7	0.1-1.6
	Infant (> 1 mo – 2 yrs)	19	0.1-3.7	13	0.1-3.2
	Child (> 2 yrs – 12 yrs)	9	0.6-3.9	7	0.3-3.2
	Adolescent (>12 – 21 yrs)	0	1.4	1	1.6
Fastidious (<i>Neisseria meningitidis</i> , <i>Neisseria sicca</i>)	Newborn (< 1 mo)	0	NA	0	NA
	Infant (> 1 mo – 2 yrs)	1	0.2	1	0.5
	Child (> 2 yrs – 12 yrs)	0	NA	0	NA
	Adolescent (>12 – 21 yrs)	1	1.1	1	0.6
Yeast (<i>Candida albicans</i> , <i>C. guilliermondii</i> , <i>C. kusei</i> , <i>C. lusitaniae</i>)	Newborn (< 1 mo)	0	NA	0	NA
	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 – 21 yrs)	1	0.2-3.0	2	2.1-2.5
Non-fermentative Gram-Negative Bacilli	Newborn (< 1 mo)	0	NA	0	NA
	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 – 21 yrs)	0	NA	0	NA
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 – 21 yrs)	6	0.5-3.5	7	0.5-3.2
<i>Staphylococcus aureus</i>	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 – 21 yrs)	2	1.5-1.7	2	1.3-1.4
<i>Enterococcus</i> 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 – 21 yrs)	8	1.5-3.1	7	1.9-2.9
<i>Streptococcus pneumoniae</i>	Newborn (< 1 mo)	0	NA	0	NA
	Infant (> 1 mo – 2 yrs)	2	1.0-2.7	1	1.1-1.6
	Child (> 2 yrs – 12 yrs)	0	NA	0	NA
	Adolescent (>12 – 21 yrs)	0	NA	0	NA
<i>Streptococcus</i> spp., Group A, B	Newborn (< 1 mo)	0	NA	0	NA
	Infant (> 1 mo – 2 yrs)	1	0.5	0	1.0
	Child (> 2 yrs – 12 yrs)	0	NA	0	NA
	Adolescent (>12 – 21 yrs)	0	NA	0	NA
Other <i>Streptococcus</i> spp.	Newborn (< 1 mo)	0	NA	0	NA
	Infant (> 1 mo – 2 yrs)	4	0.1-1.6	5	0.1-1.5
	Child (> 2 yrs – 12 yrs)	3	0.7-1.1	2	0.6-2.6
	Adolescent (>12 – 21 yrs)	1	0.7-2.4	2	1.1-2.4
Other Gram Negative*	Newborn (< 1 mo)	0	NA	0	NA
	Infant (> 1 mo – 2 yrs)	1	0.6	0	0.4
	Child (> 2 yrs – 12 yrs)	0	NA	0	NA
	Adolescent (>12 – 21 yrs)	0	NA	0	NA
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 – 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. (7), *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 ml 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*, *N. sicca*),
- ≥ 0.2 ml for Yeast (*Candida albicans*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*)

- b. Matrix comparison:*
Not Applicable
- 3. Clinical studies:
 - a. Clinical Sensitivity:*
Not Applicable
 - b. Clinical specificity:*
Not Applicable
 - c. Other clinical supportive data (when a. and b. are not applicable):*
Not Applicable
- 4. Clinical cut-off:
Not Applicable
- 5. Expected values/Reference range:

Expected percent positives will vary based on factors such as patient population, prevalence of significant organisms, site location, and contamination rates. The expected values presented below are provided based on clinical study data.

Percent positive cultures were observed to be 6.3% overall (range: 4.9% - 8.1%) and 4.1% (range: 2.5% - 6.4%) for significant isolates from three clinical sites in PF Plus culture bottles that received 0.1 ml to 4 ml of blood.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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