

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

A. 510(k) Number:

K121461

B. Purpose for Submission:

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

C. Measurand:

Aerobic bacteria and yeast

D. Type of Test:

Liquid culture medium for recovery of aerobic and facultative microorganisms (bacteria and fungi) from blood and other normally sterile body fluids.

E. Applicant:

bioMérieux, Inc.

F. Proprietary and Established Names:

BacT/ALERT[®] FA Plus Culture Bottle

G. Regulatory Information:

1. Regulation section:

21 CFR 866.2560

2. Classification:

Class I

3. Product code:

MDB

4. Panel:

83, Microbiology

H. Intended Use:

1. Intended use(s):

BacT/ALERT[®] FA Plus Culture Bottles are used with the BacT/ALERT[®] Microbial Detection System in qualitative procedures for recovery and detection of aerobic and facultative anaerobic microorganisms (bacteria and yeast) from blood and other normally sterile body fluids.

2. Indication(s) for use:

BacT/ALERT[®] FA Plus Culture Bottles are used with the BacT/ALERT[®] Microbial Detection System in qualitative procedures for recovery and detection of aerobic and facultative anaerobic microorganisms (bacteria and yeast) from blood and other normally sterile body fluids.

3. Special conditions for use statement(s):

Prescription Use Only

4. Special instrument requirements:

The BacT/ALERT[®] Microbial Detection System

I. Device Description:

The new BacT/ALERT FA Plus Culture Bottle is a modified blood culture bottle that is different from the cleared charcoal formulation BacT/ALERT FA Culture Bottle. The BacT/ALERT FA Plus Culture Bottles are used with the BacT/ALERT Microbial Detection Systems in qualitative procedures for recovery and detection of microorganisms from blood.

The predicate BacT/ALERT FA Culture Bottle contains charcoal, for its antimicrobial neutralization properties, in a complex growth medium. Charcoal has been eliminated in the BacT/ALERT FA Plus Culture Bottle, and is replaced with two types of adsorbent resins in a complex growth medium.

The BacT/ALERT FA Plus Culture Bottle was modified to optimize antimicrobial neutralization properties, and to increase the clarity of Gram stains in comparison to the predicate BacT/ALERT FA Culture Bottle.

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 or other normally sterile body fluid samples (except urine) taken from a patient suspected of having bacteremia/fungemia. 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[®] FA Culture Bottle

2. Predicate 510(k) number(s):

K020813

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	BacT/ALERT [®] FA Plus Culture Bottles are used with the BacT/ALERT [®] Microbial Detection System in qualitative procedures for recovery and detection of aerobic and facultative anaerobic microorganisms (bacteria and yeast) from blood and other normally sterile body fluids	Same
Specimen type	Human blood and normally sterile body	Same

Similarities		
Item	Device	Predicate
	fluids	
Instrumentation	BacT/ALERT Microbial Detection System	Same
Detection Technology	The system 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. If microorganisms are present, their growth produces CO ₂ and the color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow.	Same

Differences		
Item	Device	Predicate
Medium	30 ml (30 ml of complex medium and 1.6 g of adsorbent polymeric beads)	20 ml (16 ml of complex medium and 4 ml of charcoal suspension with an average density of 1.0215 g/ml)
Media ingredients	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

Differences		
Item	Device	Predicate
	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
Bottle Atmosphere	Bottle contains an atmosphere of N ₂ , O ₂ and CO ₂ under vacuum	Bottle contains an atmosphere of CO ₂ , in Oxygen under vacuum

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

1. CLSI M100-S21, Performance Standards for Antimicrobial Susceptibility Testing, Twenty-first Informational Supplement, 2011
2. CLSI) M22-A3, Quality Control for Commercially Prepared Microbiology Culture Media, 2004
3. CLSI M47-A, Principles and Procedures for Blood Cultures; Approved Guidelines, 2007
4. CLSI EP05-A2, Evaluation of Precision Performance of Clinical Chemistry Devices, Approved Guidelines, 2004
5. CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance, Approved Guideline, 2008

L. Test Principle:

If microorganisms are present in the test sample inoculated into the BacT/ALERT[®] FA Plus Culture Bottle, CO₂ will be produced when the organisms metabolize the substrates in the culture medium. 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. An analysis of the rate and amount of CO₂ increase enables the BacT/ALERT Microbial Detection System to determine if the bottle is positive, i.e., that the test sample contains viable microorganisms.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

Antimicrobial Neutralization Study

An in-house study was conducted to assess the ability of the BacT/ALERT[®] FA Plus Culture Bottle to recover and detect microorganisms in the presence of antimicrobials. In this seeding study, blood containing antimicrobials, in clinically relevant concentrations, was added directly to the BacT/ALERT[®] FA 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 FA Plus Culture Bottles. Initial testing of all antimicrobials, or combinations of antimicrobials was done at 120% PSL. If bottles fail to recover a 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 FA Plus medium based on 100% recovery of the organisms tested at the indicated percent of peak serum level tested. The effectiveness of the antimicrobials was confirmed by parallel testing using a non-neutralizing culture medium as a control.

The study demonstrated that antimicrobials from the following categories were neutralized by the BacT/ALERT[®] FA 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. The percent recovery varied by organism and drug concentration and required lowering the drug concentration to levels as low as 1% of peak serum level before observing neutralization.

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)
Daptomycin	119	Lipopeptide	<i>Staphylococcus aureus</i>	0.12 - 1	29.8	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)**
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)
			<i>Streptococcus pneumoniae</i>	0.12/2.4 - 1/19	2.7 / 31.5	100%	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)**
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)

*Initial testing concentration is calculated based on 10mL of blood containing the PSL concentration added into 30 mL of BacT/ALERT® FA Plus Culture Bottle Blood Culture Medium.

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 Study

A multi-center study was conducted to evaluate the effect of a delay, from the time the BacT/ALERT® FA Plus Culture Bottle is inoculated, to the time the bottle is placed on the instrument. This seeding study was conducted at three study sites (one in-house, and two external sites), each using a different lot of BacT/ALERT® FA 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® FA Plus Culture Bottles were tested at five different combinations of holding temperature/holding period, as indicated below, prior to be 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 a percent recovery of 100% except for culture bottles held at 35 to 37°C for 24 hours or longer before loading. At this holding temperature/period combination, the FA Plus Culture Bottles may not detect microorganisms and should be subcultured. The study results are illustrated in Table 2 below.

Table 2. Delayed Entry Study Results

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% (459/459)	14.3	8.5 - 84.0
	2-8	48	98.6% (292/296)	63.7	57.5 - 103.2
	20-25	24	98.0% (291/297)	31.8	26.2 - 74.4
	20-25	36	91.9% (272/296)	41.8	38.0 - 70.5
	35-37	8	98.9% (454/459)	16.1	10.2 - 53.8
	35-37	24	56.6% *** (259/458)	28.3	26.0 - 74.4
Negative Controls	All conditions		0.5% (1/221)**	-	-

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

**False positive observed during seeded study (1/221)

***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:*

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 3 days with a

minimum of two operators per site. Reproducibility was evaluated on each of 9 organisms. The reproducibility of the FA Plus Culture Bottles was evaluated using three different instruments (one instrument per site). Three lots of FA Plus Culture Bottles were used. For each test day/operator event, three uninoculated (negative) control bottles were incubated along with inoculated bottles (one for each inoculated repetition). Ten representative organisms were evaluated by spiking each bottle. *C. albicans* and *S. pneumoniae* were seeded into the FA 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 3 below.

Table 3. 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%			

The above data includes repeat testing performed as a result of laboratory errors at a single site (i.e. contaminated bottles/reagents, colony counts out of range, and site failure to change bottle status after positive instrument signal and positive subculture). Data excluding the laboratory errors demonstrated 100% recovery with the exception of *E. aerogenes*, which exhibited 85.0% recovery for all sites combined.

Within-Laboratory Precision (Repeatability)

A repeatability study was conducted in-house to test the ability of FA Plus culture bottles to recover and detect microorganisms in the presence of antimicrobials to which the microorganisms are susceptible. This differs from a limit of detection study, in which microorganism growth is not challenged by antimicrobials. A target inoculum level of twenty five times the LoD was used for repeatability validation. Seeded studies were conducted on multiple instruments by multiple operators. Three replicates of each organism were tested on each of three bottle lots for 12 days, resulting in a total of 108 replicates per organism per bottle type; replicates were tested for each organism/antimicrobial combination. Organisms were grown in the presence of clinically relevant concentrations of antimicrobials to which they are susceptible. In this seeded study, BacT/ALERT FA Plus bottles were subcultured at least 24 hours after being flagged positive by the instrument. Results are shown in Table 4 below.

Table 4. Summary of the Within-Laboratory Precision Data

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

b. *Linearity/assay reportable range:*

Not applicable

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

Quality Control

Quality Control was performed during the clinical study on each of 13 organisms listed in Table 5 below. Isolates were tested at least 20 test sets/day, one organism/bottle) according to a rotating schedule. Growth within 48 hours for bottles was the expected result.

Isolates were prepared via serial dilution and seeded into the FA Plus 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, contaminated and mislabeled bottles). Repeat testing resulted in acceptable results.

Table 5. 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
<i>Streptococcus pneumoniae</i>	ATCC 6305
<i>Streptococcus pyogenes</i>	ATCC 19615

Stability

The sponsor conducted studies to determine stability (shelf-life).

Three lots each of FA 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.

d. Detection limit:

Limit of Detection (LoD) performance was validated for given organisms. LoD is defined as the lowest inoculum level for which the detection proportion is 95%. Aerobic FA Plus bottles were inoculated with target inocula of 5, 30, and 100 CFU/bottle and loaded into a BacT/ALERT 3D Microbial Detection System. All testing was done in the absence of blood with the exception of 1 ml human blood for *Haemophilus influenzae*. Additional testing was performed when a LoD of approximately 5 CFU was not obtained. A minimum of thirty replicates were tested for each organism at each inoculum level.

The data was generated using bottles at end of shelf life. Bottles inoculated with *H. influenzae* received 4 ml pooled human blood supplementation. At least 95% detection was achieved at LoD. The LoDs for the organisms tested were as shown in Table 6 below.

Table 6. Summary of LoD Data

Microorganism	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

Growth Performance

An in-house study analytical was conducted to assess the ability of the BacT/ALERT® FA Plus Culture Bottle to grow and detect microorganisms. In this seeding study, multiple strains were tested for each species at target inoculum levels of 125 CFU per bottle which included blood obtained from healthy human volunteers. The actual inoculum levels ranged from 3 CFU/bottle to 298 CFU/bottle. The species listed are representatives of clinically prevalent organisms in blood. The data is shown in Table 7 below.

Table 7. Growth Performance

Microorganism	Blood			
	% Recovery (n/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 (30/30)	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</i>	100.0 (12/12)	25 - 120	12.8	11.3 - 14.4

<i>faecium</i>				
<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

The influence of potentially interfering substances was evaluated in studies conducted in-house. Seeded studies were conducted with cerebrospinal fluid, pleural fluid, synovial fluid, plasma, blood, and blood clots. Aliquots of each of these fluids also received white blood cells at concentrations relevant to bacteremia in each given body fluid. 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.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A clinical study was conducted at five study centers to evaluate the clinical performance of the BacT/ALERT FA Plus Culture Bottle using either blood or Sterile Body Fluid (SBF) samples from adult patients suspected of having either bloodstream or SBF bacterial/fungal infections.

Clinical Study (Blood Culture)

A multi-center clinical study was conducted at three different geographic sites in the U.S. comparing the performance of the FA Plus and FA blood culture bottles for aerobic culture pairs that received blood volumes between 6 ml and 10 ml (compliant pairs). A total of 1656 bottle pairs were obtained from 728 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 system. A pair of bottles was determined to have a positive status if the subculture of either the FA Plus or FA 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 FA Plus and FA culture bottles, and the ratio of FA Plus true positives to FA 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 267 isolates were recovered from all compliant aerobic culture pairs with a positive status. There were a total of 238 bottle pairs that recovered at least 1 isolate by subculture of FA Plus or FA bottles. A total of 214 bottle pairs recovered a single isolate, 19 bottle pairs recovered two isolates, and 5 bottle pairs recovered 3 isolates. The total population reported in Table 8 comprises the 267 isolates recovered from positive bottle pairs and 1418 negative bottle pairs for a total of 1685 results.

The BacT/ALERT FA Plus bottle detected a total of 208 isolates compared to the BacT/ALERT FA bottle that detected 194 isolates. Of the significant isolates, the BacT/ALERT FA Plus bottle detected a total of 159 isolates compared to the BacT/ALERT FA bottle that detected 135 isolates. Five false positives were identified by subculture of positive BacT/ALERT FA Plus bottles and comprised 0.30% (5/1685) of the study population.

The tables below compare results of the BacT/ALERT FA Plus to BacT/ALERT FA blood cultures for all compliant blood culture bottles that yielded any number of isolates on subculture (Tables 8 and 9), a single isolate alone on subculture (Table 10), and multiple isolates on subculture (Table 11).

Table 8. All compliant pairs with Single and Multiple Isolates (Blood Cultures)

Clinical Isolate Determination	Result			Total
	FA Plus=TP FA=FN	FA Plus=FN FA=TP	FA Plus=TP FA=TP	
Significant	50	26	109	185
Contaminant	18	29	18	65
Unknown	5	4	8	17
<i>Total</i>	<i>73</i>	<i>59</i>	<i>135</i>	<i>267</i>

Table 9. All Compliant Pairs with Single and Multiple Isolates Combined (Blood Cultures)

Clinical Isolate Determination	BacT/ALERT FA Plus True Positives	% of BacT/ALERT FA Plus True Positives in Population	BacT/ALERT FA True Positives	% of BacT/ALERT FA True Positives in Population	Ratio of True Positives
Significant	159	9.4% (159/1685)	135	8.0% (135/1685)	1.178
Contaminant	36	2.1% (36/1685)	47	2.8% (47/1685)	0.766
Unknown	13	0.8% (13/1685)	12	0.7% (12/1685)	1.083
<i>Total</i>	<i>208</i>	<i>12.3% (208/1685)</i>	<i>194</i>	<i>11.5% (194/1685)</i>	<i>1.072</i>

One hundred thirty five (135) isolates were detected by both FA Plus and FA, 73 isolates were detected only by FA Plus and 59 isolates were detected only by FA. The ratio of true positive rates for overall isolates was 1.072 (208/194) with a 95% CI of (0.952, 1.193). 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 10. All Compliant Pairs with Single Isolates (Blood Cultures)

Clinical Isolate Determination	FA Plus True Positives	FA True Positives	Ratio of True Positives
Significant	138	111	1.243
Contaminant	26	33	0.788
Unknown	8	9	0.889
<i>Total</i>	<i>172</i>	<i>153</i>	<i>1.124</i>

One hundred eleven (111) isolates were detected by both FA Plus and FA, 61 isolates were detected only by FA Plus and 42 isolates were detected only by FA. The ratio of true positive rates for overall single isolates was 1.124 (172/153) with a 95% CI of (0.986, 1.262).

Table 11. All Compliant Pairs with Multiple Isolates (Blood Cultures)

Clinical Isolate Determination	FA Plus True Positives	FA True Positives	Ratio of True Positives
Significant	21	24	0.875
Contaminant	10	14	0.714
Unknown	5	3	1.667
<i>Total</i>	<i>36</i>	<i>41</i>	<i>0.878</i>

Twenty four (24) isolates were detected by both FA Plus and FA, 12 isolates were detected only by FA Plus and 17 isolates were detected only by FA. The ratio of true positive rates for overall multiple isolates was 0.878 (36/41) with a 95% CI of (0.637, 1.119).

In this clinical study, there were 1413 pairs of FA Plus and FA bottles with negative instrument results for both bottles after 5 days of incubation. Among these pairs, terminal subcultures on both bottles were performed for 95 pairs, and 2 false negative results by both FA Plus and FA bottles were observed; subculture on FA Plus bottles alone was performed for 1312 pairs, and 1 false negative result was observed; both subcultures were not performed for 6 pairs of bottles. Results are summarized in Table 12 below.

Table 12. Summary of Percent False Negatives from compliant aerobic blood culture pairs that were flagged negative by the instrument for both bottles

Subculture Performed		% False Negative	
FA Plus	FA	FA Plus	FA
Yes	Yes	2.11% (2/95)	2.11% (2/95)
Yes	No	0.8% (1/1312)	N/A

Overall false negative rate for FA Plus based on a subset of terminal subcultures was 0.2% (3/1407).

Clinical Study Results (Sterile Body Fluids)

A multi-center clinical study was conducted at four different geographic sites in the U.S. and Canada comparing the performance of the FA Plus and FA culture bottles with sterile body fluid specimens (SBF). A total of 404 bottle pairs were obtained from 369 adult patients suspected of SBF bacterial/yeast infections. Sterile body fluid types evaluated were amniotic fluid, continuous ambulatory peritoneal dialysis (CAPD) fluid, cerebrospinal fluid (CSF), peritoneal fluid, pleural fluid, and synovial fluid. Clinical isolates recovered were classified as significant, contaminant, or unknown based on determination by the clinical trial sites.

A total of 92 isolates were recovered from all aerobic SBF culture pairs with a positive status. There were a total of 75 bottle pairs that recovered at least 1 isolate by subculture of FA Plus or FA bottles. A total of 62 bottle pairs recovered a single isolate, 9 bottle pairs recovered two isolates, and 4 bottle pairs recovered 3 isolates. The total population reported in Table 13 comprises the 92 isolates recovered from positive bottle pairs and 329 negative bottle pairs for a total of 421 results. The FA Plus bottle detected a total of 85 isolates compared to the FA bottle that detected 67 isolates. Of the significant isolates, the FA Plus bottle detected a total of 65 isolates compared to the FA bottle that detected 59 isolates. No false positives were observed for the FA Plus bottle from the study population (0/421).

Table 13 below compares results of the BacT/ALERT FA Plus to BacT/ALERT FA SBF cultures that yielded single or multiple isolates on subculture.

Table 13. All Pairs with Single and Multiple Isolates Combined (Sterile Body Fluids).

Clinical Isolate Determination	BacT/ALERT FA Plus True Positives	% of BacT/ALERT FA Plus True Positives in Population	BacT/ALERT FA True Positives	% of BacT/ALERT FA True Positives in Population	Ratio of True Positives
Significant	65	15.4% (65/421)	59	14.0% (59/421)	1.102
Contaminant	13	3.1% (13/421)	2	0.5% (2/421)	6.500
Unknown	7	1.7% (7/421)	6	1.4% (6/421)	1.167
<i>Total</i>	85	20.2% (85/421)	67	15.9% (67/421)	1.269

Sixty (60) isolates were detected by both FA Plus and FA, 25 isolates were detected only by FA Plus and 7 isolates were detected by only FA. The ratio of true positive rates for overall isolates was 1.269 (85/67) with a 95% CI of (1.083, 1.455). Note: A limited number of amniotic fluid (n=2) and cerebrospinal fluid specimens (n=38) were obtained during the clinical study.

Table 14 summarizes the minimum specimen volume achieved in aerobic sterile body fluid clinical study.

Table 14. BTA FA Plus Sterile Body Fluids Fill Volume (ml) – Positive Status

Specimen Type	Total No. Specimen	No. of Positives	Minimum Specimen Volume (mL)
Amniotic Fluid	2	1	1.0
CAPD (dialysis fluid)	94	26	1.0
CSF	38	4	0.1
Peritoneal Fluid	116	19	1.0
Pleural Fluid	106	17	0.5
Synovial (joint) Fluid	48	8	0.5
Total	404	75	

In this clinical study, there were 329 pairs of FA Plus and FA bottles with negative instrument results for both bottles after 5 days of incubation. Among these pairs, terminal subcultures on both bottles were performed for 297 pairs and 0 false negative results by both FA Plus and FA bottles were observed; subculture on FA Plus bottles alone was performed for 32 pairs and 0 false negative results were observed. Results are summarized in Table 15 below.

Table 15. Summary of Percent False Negatives from Aerobic SBF culture pairs that were flagged negative by the instrument for both bottles

Subculture Performed		% False Negative	
FA Plus	FA	FA Plus	FA
Yes	Yes	0.0% (0/297)	0.0% (0/297)
Yes	No	0.0% (0/32)	N/A

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):

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Expected percent positive cultures vary based on factors such as patient population, specimen type prevalence of significant organisms, site location, and contamination rates. The expected values shown below are provided based on the clinical study conducted to evaluate FA Plus Culture Bottle:

Blood Cultures: Percent positive cultures were observed to be 12.3% (range: 7.9% – 14.3%) overall and 9.4% (range: 5.0% - 11.2%) for significant isolates from three clinical trial sites in FA Plus culture bottles that received 6 ml to 10 ml of blood.

Sterile Body Fluids: Percent positive cultures were observed to be 20.2% (range: 16.4% – 24.3%) overall and 15.4% (range: 8.2% - 21.6%) for significant isolates from three clinical trial sites in FA Plus culture bottles that received sterile body fluids.

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.