510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K121455

B. Purpose for Submission:

To obtain a substantial equivalent determination for a premarket notification for the BacT/ALERT® FN Plus Culture Bottle .

C. Measurand:

Anaerobic and facultative anaerobic microorganisms.

D. Type of Test:

Liquid culture medium for recovery of microorganisms (anaerobic and facultative anaerobic) from blood and sterile body fluids using a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) that is dissolved in the culture medium.

E. Applicant:

bioMerieux, Inc.

F. Proprietary and Established Names:

BacT/ALERT® FN 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® FN Plus Culture Bottles are used with the BacT/ALERT® Microbial Detection System in qualitative procedures for recovery and detection of anaerobic and facultative anaerobic microorganisms from blood and other normally sterile body fluids.

2. Indication(s) for use:

BacT/ALERT® FN Plus Culture Bottles are used with the BacT/ALERT® Microbial Detection System in qualitative procedures for recovery and detection of anaerobic and facultative anaerobic microorganisms from blood and other normally sterile body fluids.

3. Special conditions for use statement(s):

Prescription Use Only

4. Special instrument requirements:

BacT/ALERT® Microbial Detection System

I. Device Description:

The BacT/ALERT® FN Plus Culture Bottle is a change from the cleared BacT/ALERT® FN Culture Bottle. The BacT/ALERT® FN Culture Bottles are used with the BacT/ALERT® Microbial Detection System in qualitative procedures for the recovery and detection of microorganisms from blood.

BacT/ALERT® FN Culture Bottle contains charcoal for it antimicrobial neutralization properties, in a complex growth medium. The charcoal is eliminated in the BacT/ALERT® FN Plus Culture Bottle, and is replaced with two types of adsorbent polymeric beads in the complex growth medium.

The BacT/ALERT® Microbial Detection System provides both a microbial detection

system and a culture medium bottle with suitable nutritional and environmental conditions for microorganism 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® Culture Bottles.

The BacT/ALERT® Microbial Detection System utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) that is dissolved in the 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 CO_2, 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® FN Culture Bottle

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

   K020815

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	BacT/ALERT® FN Plus Culture Bottles are used with the BacT/ALERT® Microbial Detection System in qualitative procedures for recovery and detection of anaerobic and facultative anaerobic microorganisms from blood and other normally sterile body fluids.	BacT/ALERT® FN Culture Bottles are used with the BacT/ALERT® Microbial Detection System in qualitative procedures for enhanced recovery and detection of anaerobic microorganisms from blood and other normally sterile body fluids.
Technology	Reflectance	Same
Sensor	Colorimetric/CO_2 detection	Same
Median Fill Volume	40ml	Same
Headspace Gases	Comprised of CO_2 and N_2	Same

Differences		
Item	Device	Predicate
Software	Knowledge base specifications updated to enable system to recognize the BTA FN Plus bottle type via barcodes printed on the bottles.	Knowledge base specifications in place to enable system to recognize the BTA FN bottle type via barcodes printed on the bottles.
Antibiotic Neutralization	Via resin/adsorbent polymeric beads	Via charcoal slurry
Media Ingredients	Peptones 1.48% w/v	Soybean Casein Digest 2.0 % w/v
	SPS 0.083 % w/v	SPS 0.044% w/v
	Menadione 0.00005% w/v	Menadione 0.0000625% w/v
	Hemin 0.001% w/v	Hemin 0.000625% w/v
	Pyridoxine HCL 0.0008% w/v	Pyridoxine HCL 0.001% w/v
Sample Volume Range (Blood)	Up to 10 mL	Up to 4 mL

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

1. CLSI M100-S21, Performance Standards for Antimicrobial Susceptibility Testing, Twenty-first Informational Supplement, 2011

2. Clinical and Laboratory Standards Institute (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 EP5-A2, Evaluation of Precision Performance of Clinical Chemistry Devices, Approved Guidelines, 2004

5. CLSI EP 12-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® FN Plus Culture Bottle, CO_2 will be produced when 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. 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 CO_2 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 seeded study was conducted to assess the ability of the BacT/ALERT® FN Plus Culture Bottle to recover and detect microorganisms in the presence of antimicrobials. Antimicrobials, in clinically relevant concentrations, were added directly to the BacT/ALERT® FN Plus Culture Bottles during inoculation with susceptible strains of obligate and facultative anaerobic microorganisms. The effectiveness of the antimicrobials was confirmed by parallel testing using a non-neutralizing culture medium as a control.

The study demonstrated that the following antimicrobials/antimicrobial categories were effectively neutralized such that susceptible strains of the microorganisms tested grew in the BacT/ALERT® FN Plus Culture Bottle resulting in a positive bottle signal within five days or less after inoculation: imipenem, meropenem, oxacillin, gycylcycline, marcrolides, cefoxitin, ceftaroline, aminoglycosides, fluoroquinolones, lincosamides, ketolides, and glycopeptides. Antimicrobial neutralization was not achieved for ceftazidime, ceftriaxone, or cefepime.

Less than complete neutralization was demonstrated for the following antimicrobials: cefotaxime, cefazolin, ampicillin, penicillin, and ertapenem.

The study results are illustrated in Table 1 below.

Table 1. Antimicrobial Neutralization BacT/ALERT® FN Plus Culture Bottle

Antimicrobial	PSL conc. (µg/ml)*	Antimicrobial Class	Organism	MIC (µg/ml)[1, 2, 3, 4, 5]	Initial testing conc. (µg/ml)¥	% of PSL	% Recovery (n/N)**
Ampicillin	47	b-lactam	*Clostridium perfringens*	</= 0.03 - 0.06	9.4	100%	0% (0/17)
	35.3	b-lactam	*Enterococcus faecalis*	0.5 - 2	7.1	75%	100% (17/17)
	47	b-lactam	*Escherichia coli*	2 - 8	9.4	100%	100% (17/17)

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Ampicillin + sulbactam	47 / 28	b-lactam, b-lactamase inhibitor combination	*Bacteroides fragilis*	0.5/0.25 - 2/1	9.4 / 5.6	50%	5.9% (1/17) (w/ blood)
	47 / 28	b-lactam, b-lactamase inhibitor combination	*Escherichia coli*	2/1 - 8/4	9.4 / 5.6	50%	100% (17/17) (w/ blood)
Cefazolin	47	1st generation Cephalosporin	*Escherichia coli*	1 - 4	9.4	25%	100% (17/17)
	10	1st generation Cephalosporin	*Staphylococcus aureus*	0.25 - 1	2.0	5%	100% (17/17)
Cefotaxime	2.5	3rd generation Cephalosporin	*Escherichia coli*	0.03 - 0.12	0.5	3%	52.9% (9/17)
	25	3rd generation Cephalosporin	*Staphylococcus aureus*	1 - 4	5.0	25%	100% (17/17)
	40	3rd generation Cephalosporin	*Staphylococcus aureus*	1 - 4	8.0	40%	94.1% (16/17)
Cefoxitin	132	2nd generation Cephalosporin	*Bacteroides fragilis*	4 - 16	26.4	120%	100% (18/18) (w/ blood)
	132	2nd generation Cephalosporin	*Escherichia coli*	2 - 8	26.4	120%	100% (19/19)
	110	2nd generation Cephalosporin	*Staphylococcus aureus*	1 - 4	22.0	100%	100% (17/17)
Ceftaroline	21	5th generation Cephalosporin	*Escherichia coli*	0.03 - 0.12	4.2	100%	100% (17/17)
			Staphylococcus aureus	0.12 - 0.5	4.2	100%	100% (17/17)
Clarithromycin	4.8	Macrolide	*Staphylococcus aureus*	0.12 - 0.5	0.96	120%	100% (17/17)
Clindamycin	12	Lincosamide	*Bacteroides fragilis*	0.5 - 2	2.4	120%	100% (17/17) (w/ blood)
			Staphylococcus aureus	0.06 - 0.25	2.4	120%	100% (17/17)
Daptomycin	119	Lipopeptide	*Staphylococcus aureus*	0.12 - 1	23.8	120%	100% (17/17)
Ertapenem	7.7	Carbapenem	*Bacteroides fragilis*	0.06 - 0.25	1.5	5%	100% (17/17) (w/ blood)
	115.5	Carbapenem	*Bacteroides fragilis*	0.06 - 0.25	23.1	75%	58.2% (10/17) (w/ blood)
	7.7	Carbapenem	*Staphylococcus aureus*	0.06 - 0.25	1.5	5%	100% (17/17) (w/ blood)

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	185	Carbapenem	*Staphylococcus aureus*	0.06 - 0.25	37.0	120%	70.6% (12/17)
Erythromycin Oral	2.4	Macrolide	*Staphylococcus aureus*	0.25 - 1	0.5	120%	100% (17/17)
Gentamicin	12	Aminoglycoside	*Escherichia coli*	0.25 - 1	2.4	120%	100% (17/17)
Imipenem	48	Carbapenem	*Bacteroides fragilis*	0.03 - 0.125	9.6	120%	100% (19/19) (w/ blood)
	48	Carbapenem	*Enterococcus faecalis*	0.5 - 2	9.6	120%	100% (18/18)
	48	Carbapenem	*Staphylococcus aureus*	0.015 - 0.06	9.6	120%	100% (17/17)
Levofloxacin	10.3	Fluoroquinolone	*Enterococcus faecalis*	0.25 - 2	2.1	120%	100% (17/17)
Meropenem	49	Carbapenem	*Bacteroides fragilis*	0.03 - 0.25	9.8	100%	100% (22/22)
			Staphylococcus aureus	0.03 - 0.12	9.8	100%	52.9% (9/17) (w/ blood)
Moxifloxacin	4.5	Fluoroquinolone	*Escherichia coli*	0.008 - 0.06	0.9	100%	100% (17/17)
	5.4	Fluoroquinolone	*Bacteroides fragilis*	0.125 - 0.5	1.1	120%	100% (17/17)
Ofloxacin	4.6	Fluoroquinolone	*Staphylococcus aureus*	0.12 - 1	0.92	100%	100% (17/17)
Oxacillin	18	b-lactam	*Peptoniphilus asaccharolyticus*	N/A	3.6	120%	100% (17/17) (w/ blood)
			Staphylococcus aureus	0.12 - 0.5	3.6	120%	100% (17/17)
Penicillin G	10	b-lactam	*Clostridium perfringens*	0.063 - 0.27	2.0	50%	5.9% (1/17) (w/ blood)
	24	b-lactam	*Streptococcus pneumoniae*	0.25 - 1	4.8	120%	100% (17/17) (w/ blood)
Piperacillin + Tazobactam	188.2 / 20.8	b-lactam, b-lactamase inhibitor combination	*Staphylococcus aureus*	0.25/4 - 2/4	37.6 / 3.8	100%	100% (17/17) (w/ blood)
	228 / 22.8	b-lactam, b-lactamase inhibitor combination	*Bacteroides fragilis*	0.125/4 - 0.5/4	45.6 / 4.6	120%	100% (17/17) (w/ blood)
Telithromycin	2.3	Macrolide	*Staphylococcus aureus*	0.06 - 0.25	0.46	100%	100% (17/17)

Table 1. Antimicrobial Neutralization BacT/ALERT® FN Plus Culture Bottle							
Antimicrobial	**PSL conc. (µg/ml)***	**Antimicrobial Class**	**Organism**	**MIC (µg/ml)[1, 2, 3, 4, 5]**	**Initial testing conc. (µg/ml)¥**	**% of PSL**	**% Recovery (n/N)****
Tigecycline	0.63	Glycylcycline	*Escherichia coli*	0.03 - 0.25	0.13	100%	94.1% (16/17)
			Streptococcus pneumoniae	0.015 - 0.12	0.13	100%	100% (17/17)
	0.76	Glycylcycline	*Staphylococcus aureus*	0.03 - 0.25	0.15	120%	100% (17/17)
Tobramycin	12	Aminoglycoside	*Escherichia coli*	0.25 - 1	2.4	120%	100% (17/17)
Trimethoprim / sulfamethoxazole	11 / 126	Sulfonamide combination	*Streptococcus pneumoniae*	0.12/2.4 - 1/19	2.2 / 25.2	120%	100% (34/34)
Vancomycin	50	Glycopeptide	*Clostridium perfringens*	1	10.0	100%	100% (17/17) (w/ blood)
			Staphylococcus aureus	0.5 - 2	10.0	120%	100% (17/17)

CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S22. Wayne, Pa; Clinical and Laboratory Standards Institute; 2012.

Carman RJ and TD Wilkins. 1991. In vitro susceptibility of rabbit strains of Clostridium spiroforme to antimicrobial agents. Veterinary Microbiology. 28:391-397

Stevens DL, K A Maier and J E Mitten. 1987. Effect of antibiotics on toxin production and viability of Clostridium perfringens. Antimicrob. Agents Chemother.31: 213-218.

Zurenko G E, B H Yagi, R D Schaadt, J W Allison, J O Kilburn, S E Glickman, D K Hutchinson, M R Barbachyn and S J Brickner. 1996. In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. Antimicrob. Agents Chemother. 40: 839-845.

Goldstein, E.J.C, D. M. Citron, C. V. Merriam, Y. A. Warren, K. L. Tyrell, and H. T. Fernandez. 2003. In vitro activities of daptomycin, vancomycin, quinupristin-dalfopristin, linezolid, and five other antimicrobials against 307 Gram-positive anaerobic and 31 Corynebacterium clinical isolates. Antimicrob. Agents Chemother. 47:337-341.

* Antimicrobial Therapy Inc. 2009 Sanford guide to antimicrobial therapy. Sperryville, VA.
** Growth of susceptible microorganism in the presence of antimicrobial
¥ Initial concentration achieved by the addition of 10mL of blood containing the %Peak Serum Level (PSL) of antibiotic into 30mL of the BacT/ALERT® FN Plus culture medium.

Delayed Entry Study

A study was conducted to evaluate the effect of a delay, from the time the BacT/ALERT® FN Plus Culture Bottle is inoculated, to the time the bottle is

placed on the instrument. The study was conducted at three study sites (one in-house, and two external sites), each using a different lot of BacT/ALERT® FN Plus Culture Bottles. Three replicates each of six isolates, namely, *S. aureus, E. coli, S. pneumoniae, E. faecium, B. fragilis and C. perfringenes*, were tested using an inoculum range from 41-253 CFU/bottle. In addition to the bacterial isolates, each BacT/ALERT® FN Plus Culture Bottle was inoculated with 3-4 mLs of blood from healthy volunteers. BacT/ALERT® FN 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. The study results are illustrated in Table 2 below.

- 2-8ºC for 48 hours
- 20-25ºC for 24 hours
- 20-25ºC for 36 hours
- 35-37ºC for 8 hours
- 35-37ºC for 24 hours

The study demonstrated that all holding conditions showed a percent recovery of 100% with the exception of those held at 35-37ºC for 24 hours, which demonstrated a percent recovery of 80%. The decreased recovery of 80% was due to 16 culture bottles seeded with isolates (4 isolates of *E. faecium*, and 12 isolates of *S. pneumonia*e) that were not flagged positive by the BacT/ALERT® instrument. The BacT/ALERT® FN Plus Culture Bottle may not detect microorganisms if held at 35-37ºC for 24 hours before loading and should therefore be subcultured.

Table 2. Delayed Entry Study

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% (89/89)	15.9	9.5 - 52.8
	2-8	48	100.0% (65/65)	63.3	50.1 - 90.4
	20-25	24	100.0% (62/62)	34.7	26.0 - 79.2
	20-25	36	100.0% (62/62)	43.6	38.0 - 78.6
	35-37	8	100.0% (72/72)	17.7	10.0 - 53.4
	35-37	24	80.0%¥ (64/80)	28.6	26.0 - 52.3
Negative Controls	All conditions		0.0% (0/51)	-	-

¥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:

Reproducibility

A reproducibility study was conducted to evaluate the BacT/ALERT® FN Plus Culture Bottle. The study was conducted at three sites (one internal and two external), for three days, with two operators, using three different instruments, and three reagent lots. Eight microorganisms representative of those most commonly associated with blood stream infection were seeded into the blood culture bottles at inoculum levels ranging from 1 to 500 CFU/bottle, depending on the microorganism. An initial total of 144 replicates were tested at each study site. Percent recovery reflects those instances where the inoculated culture bottle was flagged positive by the instrument and Gram stain/subculture were consistent with the seeded organism. Table 3 provides a summary of the study results. The data in Table 3 includes repeat testing performed due to a number of technical errors (i.e. contaminated culture bottles/reagents, colony counts out of range and failure to change bottle status after positive instrument signal and positive subculture). Data excluding the technical errors demonstrated 100% recovery for all isolates with the exception of *E. aerogenes* which exhibited 96.3% recovery for all sites combined.

Table 3. Reproducibility Study

Sample Input	**% Recovery**				**Time to Detection**		**CFU/ Bottle**
	Site 1	**Site 2**	**Site 3**	**Overall**	**Mean**	**Range**	
Staphylococcus aureus	96.3% (26/27)	79.2% (19/24)	100.0% (33/33)	92.9% (78/84)	20.2	18.5-35.7	2-12
Escherichia coli	100.0% (18/18)	79.2% (19/24)	100.0% (33/33)	93.3% (70/75)	12.8	11.4-20.8	2-11
Enterococcus faecalis	100.0% (30/30)	83.3% (20/24)	97.0% (32/33)	94.3% (82/87)	24.6	17.9-30.4	2-15
Clostridium perfringens	100.0% (18/18)	96.8% (61/63)	100.0% (30/30)	98.2% (109/111)	12.2	10.2-17.3	3-122
Enterobacter aerogenes	90.0% (27/30)	75.0% (18/24)	90.5% (38/42)	86.5% (83/96)	14.6	11.9-16.7	1-270
Listeria monocytogenes	100.0% (21/21)	100.0% (24/24)	100.0% (33/33)	100.0% (78/78)	22.8	20.6-37.0	1-13
Salmonella enterica	100.0% (24/24)	79.2% (19/24)	100.0% (30/30)	93.6% (73/78)	13.3	12.4-14.4	1-16
Streptococcus pneumoniae	100.0% (30/30)	100.0% (36/36)	100.0% (18/18)	100.0% (84/84)	17.5	13.3-23.1	5-500
Overall	**98.0% (194/198) 95% CI: 94.9%, 99.5%**	**88.9% (216/243) 95% CI: 84.3%, 92.6%**	**98.0% (247/252) 95% CI: 95.4%, 99.4%**	**94.8% (657/693) 95% CI: 92.9%, 96.3%**			

Within-Laboratory Precision (Repeatability) Study

An in-house seeded study was conducted to evaluate the BacT/ALERT® FN Plus Culture Bottle's ability to repeatedly recover and detect microorganisms in the presence of clinically relevant concentrations of antimicrobials to which the microorganisms are susceptible, within five days after bottle inoculation. The study was conducted over a period of 12 days, using multiple instruments, by a minimum of two operators. A minimum of 108 replicates were tested for each antimicrobial/organism combination. In the conduct of the study, the BacT/ALERT® FN Plus culture bottles were subcultured at least 24 hours after being flagged positive by the instrument. Table 4 illustrates the study results.

Table 4. Within-Laboratory Precision (Repeatability) Study

Sample Input		CFU/ bottle (range)	% Recovery				Time to Detection (hours)	
Organism	**Antimicrobial**		**Lot 1**	**Lot 2**	**Lot 3**	**Overall**	**Mean**	**Range**
B. fragilis	Imipenem	136 - 406	100.0	100.0	100.0	100.0	36.9	30.2 – 55.2
C. perfringens	Vancomycin	75 - 204	100.0	94.4	100.0	98.2	14.5	11.1 – 22.0
S. aureus	Oxacillin	94 - 158	100.0	100.0	100.0	100.0	17.7	15.1 – 24.3

b. Linearity/assay reportable range:

Not applicable

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

Quality Control

Quality control (QC) was performed during the clinical trial using each of the three organisms listed in Table 5 below. Isolates (one organism per bottle) were tested according to a rotating schedule. Growth within 5 days after inoculation was the expected result.

Serial dilutions of each isolate was prepared and seeded into the BacT/ALERT® FN Plus Culture Bottle at a target inoculum of 100 CFU/bottle with an acceptable range of 30-300 CFU/bottle. Overall QC results were found to be acceptable. Instances where unacceptable QC 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. Quality Control Organisms (Anaerobic)	
Bacteroides fragilis	ATCC 25282
Bacteroides vulgatus	ATCC 8482
Clostridium perfringens	ATCC 13124

Stability (Shelf-life)

Studies were conducted to determine the stability (shelf-life) of the BacT/ALERT® FN Plus Culture Bottle. Four lots of BacT/ALERT® FN Plus culture bottles for design verification and three lots for product validation were tested until they were 13 months old. At the beginning of the study, bottles were thermally stressed, or not thermally stressed, and then stored at either 15-30 ºC or 2-8 ºC for a total of four 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 of blood, and if coagulation was prevented when direct blood draw was allowed to continue until the bottle vacuum was exhausted.
Initial stability studies were conducted using a high target inoculum in comparison to the limit of detection (LoD). Subsequent studies were conducted using levels of organisms around the LoD using culture bottles at end of shelf life, which demonstrated that stability can be achieved for the claimed shelf life.

d. Detection limit:

The limit of detection (LoD) for the BacT/ALERT® FN Plus Culture Bottle was determined using a panel of microorganisms listed in Table 6 below. LoD is defined as the lowest inoculum level for which the detection proportion is 95%. BacT/ALERT® FN Plus culture bottles were inoculated with target microorganism concentration of 5 CFU/bottle and loaded into a BacT/ALERT® 3D Microbial Detection System (BTA). All testing was done in the absence of blood with the exception of 1 mL of blood for *B. fragilis* and *S. pneumoniae*. A minimum of thirty replicates were tested for each organism. At least 95% detection was achieved for all isolates tested at LoD. The LoD values for the microorganisms tested are listed in Table 6 below.

Table 6. Limit of Detection		
Microorganism	Strain Identification	LoD (CFU/bottle)
Bacteroides fragilis	ATCC 25285	5
Clostridium perfringens	NCTC 8798	4
Enterobacter aerogenes	ATCC 13048	8
Enterococcus faecalis	NCTC 12697	4
Escherichia coli	NCTC 12923	4
Listeria monocytogenes	ATCC 15313	6
Salmonella enterica	ATCC 14028	5
Staphylococcus aureus	NCTC 10788	4
Streptococcus pneumoniae	ATCC 6305	6

Analytical Sensitivity (Growth Performance)

The data in Table 7 below 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 per bottle. Actual inoculum levels ranged from 3 CFU/bottle to 299 CFU/bottle. In this seeded study BacT/ALERT® FN Plus 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 and sterile body fluids.

Table. 7. Growth Performance

Microorganism	Blood				No Blood			
	% Recovery (n)	Range CFU/bottle	Time to Detection (hours)		% Recovery* (n=3)	Range CFU/bottle	Time to Detection (hours)	
			Mean	Range			Mean	Range
Staphylococcus aureus	100.0 (15/15)	54 - 150	14.6	12.9 – 16.7	100	116 - 150	21.8	21.3 – 22.0
Escherichia coli	100.0 (15/15)	73 - 254	10.9	10.4 – 12.4	100	73 - 176	11.6	10.4 – 12.9
Bacteroides fragilis	100.0 (18/18)	9 - 154	29.7	24.3 - 43.6	66.7 (2/3)	19 - 154	97.2	79.2 - 115.2
Streptococcus pneumoniae	100.0 (15/15)	4 - 260	16.5	11.5 - 43.8	100	4 - 25	17.5	16.0 – 19.3
Clostridium perfringens	100.0 (18/18)	58 - 210	17.3	12.0 - 40.1	100.0 (8/8)	76 - 210	27.1	14.1 - 35.7
Klebsiella pneumoniae	100.0 (15/15)	89 - 123	11.2	10.4 - 13.1	100	95 - 123	12.6	12.1 - 13.4
Fusobacterium nucleatum	70.6 (12/17)	19 - 204	74.4	36.0 - 108.0	75.0 (3/4)	19 - 116	56.1	41.2 - 64.8
Streptococcus agalactiae	100.0 (15/15)	14 - 194	16.9	12.7 - 28.9	100	21 - 34	25.9	20.5 - 33.4
Enterococcus faecalis	100.0 (15/15)	63 - 259	13.7	11.9 - 19.4	100	71 - 169	22.4	17.8 - 24.8
Parvimonas micra	80.0 (16/20)	46 - 154	51.4	37.3 - 69.6	0.0 (0/4)	46 - 154	-	-
Enterobacter cloacae	100.0 (15/15)	111 - 200	11.9	11.1 - 12.5	100	111 - 185	13	11.9 - 14.7
Proteus mirabilis	100.0 (15/15)	36 - 213	11.4	10.9 - 12.5	100	36 - 213	11.9	11.5 - 12.7
Eggerthella lenta	86.7 (13/15)	83 - 175	41	34.8 - 60.0	66.7 (2/3)	83 - 151	46	44.0 - 48.0
Staphylococcus epidermidis	100.0 (15/15)	44 - 135	21	17.4 - 25.3	100	44 - 105	29.3	24.5 - 36.8
Listeria monocytogenes	100.0 (15/15)	121 - 251	17.1	15.5 - 19.3	100	121 - 251	19.2	17.7 - 20.3
Clostridium tertium	100.0 (15/15)	24	12.5	11.4 – 13.5	100	24	14.8	14.1 – 16.0
Clostridium septicum	50.0 (20/40)	25 - 146	31.7	13.9 – 62.4	40.0 (2/5)	90 - 146	43.4	17.1 – 69.6
Peptoniphilus asaccharolyticus	60.0 (12/20)	49 - 296	50.9	34.4 – 79.2	25.0 (1/4)	81 - 296	44.7	-

Less than 100% detection was observed for some species, to include *Capnocytophaga ochracea*, *Cardiobacterium hominis*, *Haemophilus parainfluenzae*, and *Granulicatella adiacens*.

*In case of less than 100% recovery, it is recommended to add blood such as sterile defibrinated horse blood (10% v/v).

e. Analytical specificity:

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:

Clinical Study Results (Blood Cultures)

A multi-center clinical study was conducted at three different geographic sites in the U.S. comparing performance of the BacT/ALERT® FN Plus (FN Plus) and BacT/ALERT® FN (FN) blood culture bottles for anaerobic culture pairs that received blood volumes between 6 ml and 10 ml (compliant pairs). A total of 2514 anaerobic bottle pairs were obtained from 1080 adult patients suspected of blood stream bacterial/yeast infections. Subcultures of both bottles were performed for any bottle in the set determined to be positive by the BacT/ALERT® system. A pair of bottles was determined to have a positive status if subculture of either the FN Plus or FN 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 FN Plus and FN culture bottles and the ratio of FN Plus true positives to FN true positives were 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 312 isolates were recovered from all compliant anaerobic blood culture pairs with a positive status. There were a total of 289 bottle pairs that recovered at least 1 isolate by subculture of FN Plus or FN bottles. A total of 266 bottle pairs recovered a single isolate and 23 bottle pairs recovered two isolates. The total population reported in Table 8 comprises the 312 isolates recovered from positive

bottle pairs and 2225 negative bottle pairs for a total of 2537 results. The FN Plus bottle detected a total of 282 isolates compared to the FN bottle that detected 192 isolates. Of the significant isolates, the FN Plus bottle detected a total of 202 isolates compared to the FN bottle that detected 150 significant isolates. Three false positives were identified on subculture of positive FN Plus bottles and comprised 0.12% (3/2537) of the study population.

The tables below compare results of the BacT/ALERT® FN Plus to BacT/ALERT® FN blood cultures for all compliant blood culture bottles that yielded any number of isolates on subculture (Table 8), a single isolate alone on subculture (Table 9), and multiple isolates on subculture (Table 10). For greater detail relative to the clinical study design and calculations of confidence intervals, refer to the publication, *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 8. All Compliant Pairs with Single and Multiple Isolates Combined (Blood Cultures)

Clinical Determination	FN Plus True Positives	% of FN Plus True Positives in Population	FN True Positives	% of FN True Positives in Population	Ratio of True Positives
Significant	202	8.0% (202/2537)	150	5.9% (150/2537)	1.347
Contaminant	58	2.3% (58/2537)	30	1.2% (30/2537)	1.933
Unknown	22	0.9% (22/2537)	12	0.5% (12/2537)	1.833
Total	*282*	*11.1% (282/2537)*	*192*	*7.6% (192/2537)*	1.469

One hundred sixty two (162) isolates were detected by both FN Plus and FN, 120 isolates were detected only by FN Plus and 30 isolates were detected only by FN.

The ratio of true positive rates for overall isolates was 1.469 (282/192) with a 95% CI of (1.317, 1.621).

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

Clinical Determination	FN Plus True Positives	FN True Positives	Ratio of True Positives*
Significant	177	133	1.331
Contaminant	51	27	1.889
Unknown	15	9	1.667
Total	*243*	*169*	1.438

*One hundred forty six (146) isolates were detected by both FN Plus and FN, 97 isolates were detected only by FN Plus and 23 isolates were detected only by FN.

The ratio of true positive rates for overall single isolates was 1.438 (243/169) with a 95% CI of (1.286, 1.590).

Table 10. All Compliant Pairs with Multiple Isolates (Blood Cultures)

Clinical Determination	FN Plus True Positives	FN True Positives	Ratio of True Positives*
Significant	25	17	1.471
Contaminant	7	3	2.333
Unknown	7	3	2.333
Total	*39*	*23*	1.696

*Sixteen (16) isolates were detected by both FN Plus and FN, 23 isolates were detected only by FN Plus and 7 isolates were detected only by FN. The ratio of true positive rates for overall multiple isolates was 1.696 (39/23) with a 95% CI of (1.088, 2.304).

In this clinical study, there were a total of 2218 compliant anaerobic blood culture pairs of FN Plus and FN bottles with negative instrument results for both bottles after 5 days of incubation. Among these pairs, terminal subcultures on both bottles were performed for 63 pairs and 7 false negative results by both FN Plus and FN bottles were observed; subculture of FN Plus bottles alone was performed for 844 pairs and 4 false negative results were observed; both subcultures were not performed for 1307 pairs of bottles. Results are summarized in Table 11 below.

Table 11. Summary of Percent False Positive from compliant Anaerobic blood culture pairs that were flagged negative by instrument for both bottles

Subculture Performed FN Plus	***Subculture Performed FN***	***% False Negative FN Plus***	***% False Negative FN***
Yes	***Yes***	***11.1% (7/63)****	***11.1% (7/63)***
Yes	***No***	***0.5% (4/844)***	***NA***

*Of these 7 positive subcultures, 2 subcultures yielded isolates that are strict aerobes (*Acinetobacter baumannii, Pseudomonas aeruginosa*). The FN Plus Bottle is not intended to detect strict aerobes from blood or other normally sterile body fluids.
Overall false negative rate for FN Plus based on a subset of terminal subcultures was 1.2% (11/907) and excluding strict aerobes was 1.0% (9/907)

A comparative yield of microorganisms (number of isolates) recovered on subculture of FN Plus and FN cultures are presented in Table 12 below.

Table 12. Comparative Yield of Microorganisms from BacT/ALERT® FN Plus and BacT/ALERT® FN (number of isolates) – Blood Cultures

Group	BacT/ALERT® FN Plus	BacT/ALERT® FN
Anaerobes*	10	4
Enterobacteriaceae	50	43
Enterococcus spp.	28	19
Yeasts	0	0
Other Gram-Negative	6	3
Other Gram-Positive	9	6
Coagulase-Negative Staphylococcus	93	53
Staphylococcus aureus	65	42
Streptococcus spp.	21	22

Note: Isolate table includes polymicrobic cultures.

*Anaerobic isolates recovered in clinical trial: *Bacteroides caccae (4), Bacteroides fragilis (2), Lactobacillus spp. (1), Peptostreptococcus micros (1), Peptostreptococcus spp (1), Prevotella denticola (1), Propionibacterium acnes (1), Propionibacterium spp.*

Clinical Study Results – (Sterile Body Fluid Cultures)

A multi-center clinical study was conducted at four different geographic sites in the U.S. and Canada comparing the performance of the BacT/ALERT® FN Plus (FN Plus) and BacT/ALERT® FN (FN) culture bottles with sterile body fluid specimens (SBF). A total of 339 bottles pairs were obtained from 310 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 77 isolates were recovered from all anaerobic SBF culture pairs with a positive status. There were a total of 61 bottle pairs that recovered at least 1 isolate by subculture of FN Plus or FN bottles. A total of 50 bottle pairs recovered a single isolate, 7 bottle pairs recovered two isolates, 3 bottle pairs recovered 3 isolates, and 1 bottle pair recovered 4 isolates. The total population reported in Table 8 comprises the 77 isolates recovered from positive bottle pairs and 278 negative bottle pairs for a total of 355 results. The FN Plus bottle detected a total of 72 isolates compared to the FN bottle that detected 59 isolates. Of the significant isolates, the FN Plus bottle detected a total of 52 isolates compared to the FN bottle that detected 50 isolates. No false positives were identified for the FN Plus bottle from the study population (0/355).

Table 13 below compares results of the BacT/ALERT® FN Plus to BacT/ALERT® FN SBF cultures that yielded single or multiple isolates on subculture. For greater detail relative to the clinical study design and calculations of confidence intervals, refer to the publication, *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 13. All Pairs with Single and Multiple Isolates Combined (Sterile Body Fluid Cultures)

Clinical Determination	FN Plus True Positives	% of FN True Positives in Population	FN True Positives	% of FN True Positives in Population	Ratio of True Positives
Significant	52	14.6% (52/355)	50	14.1% (50/355)	1.040
Contaminant	12	3.4% (12/355)	2	0.6% (2/355)	6.000
Unknown	8	2.2% (8/355)	7	2.0% (7/355)	1.143
Total	*72*	*20.3% (72/355)*	*59*	*16.6% (59/355)*	1.220

Fifty four (54) isolates were detected by both FN Plus and FN, 18 isolates were detected only by FN Plus and 5 isolates were detected only by FN. The ratio of true positive rates for overall isolates was 1.220 (72/59) with a 95% CI of (1.044, 1.396).

Table 14 below summarizes the minimum specimen volume achieved in anaerobic SBF clinical trials.

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

Specimen Type	Total No. Specimen	No. of Positives	Minimum Specimen Volume
CAPD	75	22	1.0
CSF	24	2	0.1
Peritoneal Fluid	106	16	0.3
Pleural Fluid	93	22	0.2
Synovial (joint) Fluid	41	10	0.2
Total	339	72	

In this clinical study, there were 278 pairs of FN Plus and FN bottles with negative instrument results for both bottles after 5 days of incubation. Among these pairs, terminal subcultures on both bottles were performed for 166 pairs and 11 false negative results by both FN Plus and FN bottles were observed. Subculture on FN Plus bottles alone was performed for 112 pairs and 0 false negative results were observed. Results are summarized in Table 15 below.

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

Subculture Performed FN Plus	***Subculture Performed FN***	***% False Negative FN Plus***	***% False Negative FN***
Yes	***Yes***	***6.6% (11/166)****	***6.6% (11/166)****
Yes	***No***	***0.0% (0/112)***	***N/A***

* Of these 11 positive subcultures, 8 subcultures yielded isolates that were strict aerobes (2 *Acinetobacter baumannii, 1 Candida albicans*, 2 *Candida parapsilosis*, 1 *Cryptococcus neoformans*, 1 *Micrococcus spp., 1 Pseudomonas aeruginosa*).
The FN Plus Bottle is not intended to detect strict aerobes from blood or other normally sterile body fluids.
Overall false negative rate for FN Plus based on a subset of terminal subcultures is 4.0% (11/278) and excluding strict aerobes is 1.1% (3/278)

A comparative yield of microorganisms (number of isolates) recovered on subculture of FN Plus and FN cultures are presented in Table 16 below.

Comparative Yield of Microorganisms from BacT/ALERT® FN Plus and BacT/ALERT® FN (number of isolates) – Sterile Body Fluid Cultures

Table 16.

Group	**BacT/ALERT® FN Plus**	**BacT/ALERT® FN**
Anaerobes*	6	6
Enterobacteriaceae	9	8
Enterococcus spp.	14	11
Yeasts	4	2
Other Gram-Negative	2	1
Other Gram-Positive	1	0
Coagulase-Negative Staphylococcus	19	16
Staphylococcus aureus	9	9
Streptococcus spp.	8	6

Note: Isolate table includes polymicrobic cultures.

*Anaerobes recovered in clinical trial: *Bacteroides fragilis (2: 1 FN Only; 1 FN Plus & FN),Clostridium ramosum (1 FN Plus & FN), Clostridium spp. (1 FN Plus & FN), Prevotella loescheii (1 FN Plus & FN), Prevotella melaninogenica (1 FN Plus Only), Veillonella spp. (1 FN Plus & FN)*

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

6. Expected values/Reference range:

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

Percent positive cultures were observed to be 11.1% (range: 8.7%-14.3%) overall and 8.0% (range: 6.4% - 9.3%) for significant isolates from three clinical trial sites in FN Plus culture bottles that received 6 ml to 10 ml of blood.

Percent positive cultures were observed to be 20.3% (range: 17.0%-25.3%) overall and 14.6% (range: 6.1% - 24.1%) for significant isolates from three clinical trial sites in FN 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.