A COMPARATIVE PERFORMANCE STUDY OF ADJUSTED BACT/ALERT® iFA PLUS FOR GROWTH PROMOTION AND ANTIMICROBIAL NEUTRALIZATION

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INTRODUCTION

Automated culture systems are widely used for rapid-real-time microbial contamination detection for pharmaceutical products including small molecule, bioproduction, and cell and gene therapy (CAGT)[1,2,3]. CAGT manufacturers have widely adopted automated growth-based technologies as an alternative to the compendial USP <71> sterility test or by following the compendial EP 2.6.27 that includes automated growth-based methods for detection of contaminating microorganisms[4,5]. These therapies may contain residual antimicrobials from cell culture media, biopsy and tissue transport media, and cryoprotectants to mitigate contamination. The BACT/ALERT® 3D (BTA) Microbial Detection System provides an automated non-destructive growth-based rapid microbial alternative to the 14-day traditional test method and is capable of detecting a variety of aerobic and anaerobic microorganisms including fungi[6]. The BTA method utilizes direct inoculation of ≤ 10 mL of product into the BTA culture bottle consisting of supplemented Tryptic Soy Broth along with atmospheric conditions required for aerobic or anaerobic growth. The BTA culture bottles are available standard and with resin to neutralize residual antibiotics present in the sample.

The BACT/ALERT® iFA Plus (iFA Plus) is a neutralizing culture medium used with the BACT/ALERT® 3D as an non-destructive rapid microbiological method. The iFA Plus medium was recently adjusted through the addition of trace elements to extend shelf-life from 10 months to 12 months from manufacturing date; and achieve a faster time-to-detection (TTD) for specific *Candida* species.

OBJECTIVE

We performed a series of studies to determine the impact of the addition of trace elements on growth promotion as well as the ability to neutralize a range of antimicrobials. Growth promotion testing was performed comparing the adjusted and predicate bottle using ICH sterility microorganisms and a range of relevant industrial microorganisms in the presence and absence of antimicrobials. The performance of the adjusted iFA Plus was also compared against the BACTEC™ Plus Aerobic/F bottles in the presence of commonly used antimicrobial agents.

MATERIALS AND METHODS

The ability of adjusted BTA iFA Plus to support growth of microorganisms in the presence and absence of antimicrobial agents was tested vs. its predicate and Becton Dickinson (BD) BACTEC™ Plus Aerobic/F bottles. Both contain adsorbent polymeric beads, i.e. resins, for neutralizing antimicrobial agents. BTA iAST and BACTEC Standard Aerobic/F bottles were used as positive and efficacy controls.

Bottles were seeded with inocula prepared from culture or from BIOBALL™ and/or BIOBALL® MULTISHOT-550 at less than <100 CFU in a 0.5 mL inoculum suspension. A portion of the inoculum suspension was plated in duplicate on culture media plates depending on microorganism (TSA, SDA or R2A) to calculate the actual CFU delivered per bottle.

Un-inoculated bottles were included as negative controls. Predicate BTA iFA Plus, adjusted BTA iFA Plus and iAST bottles were incubated in the BACT/ALERT DUAL-T instrument at both 22.5°C and 32.5°C. BACTEC Plus Aerobic/F and Standard Aerobic/F bottles were incubated in a BACTEC FX instrument at 35°C. Mean Time to Detection (TTD) data for each microorganism was compared between the various media tested.

Growth Promotion: Nineteen(19) microbial genera recommended by the ICH and pharmaceutical environmental isolates were evaluated using nine (9) bottle replicates per microorganism. The adjusted iFA Plus culture bottle was considered equivalent to or improved vs. the predicate iFA Plus if the upper bound of the 95% confidence interval around the median TTD meets or exceeds -1 hour or -10% per the rule: if the iFA Plus median TTD exceeds 10 hours, use -10%; otherwise use -1 hour.

Neutralization: Concentrated stock solutions of each drug were prepared, sterile filtered, and injected into the BTA media using <1 mL to minimize any dilution effect prior to microorganism inoculation (Table 1). A minimum of five (5) replicates per bottle type, per temperature, per drug/microorganism pair were performed. Controls were inoculated in triplicate. Negative test bottles were examined visually and subcultured to confirm absence of growth.

Table 1. Antimicrobial neutralization test panel used for evaluating the neutralization capacity of adjusted iFA Plus

Microorganism	Antimicrobial	Concentration
A. brasiliensis NCPF 2275	Amphotericin B	10 µg Amphotericin B
	Cocktail + Penase1	500 IU Penicillin, 500 µg Streptomycin, 10 µg Amphotericin B
C. albicans NCPF 3179	Amphotericin B	10 µg Amphotericin B
	Cocktail + Penase1	500 IU Penicillin, 500 µg Streptomycin, 10 µg Amphotericin B
B. subtilis NCTC 10400	Amikacin	300 µg Amikacin
	Streptomycin	500 µg Streptomycin
	Vancomycin	200 µg Vancomycin
	Penicillin G with Penase1	500 IU Penicillin
	Penicillin G without Penase	24 IU Penicillin
	Cocktail+ Penase1	500 IU Penicillin, 500 µg Streptomycin, 10 µg Amphotericin B
S. aureus NCTC 10788	Amikacin	300 µg Amikacin
	Streptomycin	1000 µg Streptomycin
	Vancomycin	200 µg Vancomycin
	Penicillin G with Penase1	500 IU Penicillin
	Penicillin G without Penase	50 IU Penicillin
	Cocktail+ Penase1	500 IU Penicillin, 500 µg Streptomycin , 10 µg Amphotericin B

Penase: For antimicrobial combinations that include Penase, 0.1 mL of concentrated Penase will be added to the bottle

RESULTS AND DISCUSSION

Growth Performance: Both the iFA Plus and adjusted iFA Plus bottle had 100% recovery (9/9 replicates) for a total of 26 microorganisms (20 bacteria, 2 yeasts, and 4 molds) representing 19 genera (Table 2). The TTD was determined to be statistically equivalent for 23/26 organisms at 22.5°C and for 19/26 organisms at 32.5°C. *Aureobasidium pullulans, Kocuria rhizophilia,* and *Micrococcus luteus* had significantly improved TTD in the adjusted formulation, at both 22.5°C and 32.5°C. Three (3) microorganisms had significantly longer TTD in the adjusted formulation at 32.5°C. However, when longer at 32.5°C, the same strain was equivalent or better when tested at 22.5°C.

RESULTS AND DISCUSSION (CONTINUED)

Table 2. Time-to-detection (in days) of Industry relevant organisms in iFA Plus and adjusted iFA Plus

Microorganism	Incubation Temperature (°C)	CFU	Median TTD (Days)	
			iFA Plus	Adjusted iFA Plus
Yeast & Mold				
Aspergillus brasiliensis NCPF 2275	22.5	24	4.73	4.31
	32.5		2.23	2.10
Aspergillus fumigatus 0411768	22.5	7	8.00	6.35
	32.5		2.19	2.23
Aureobasidium pullulans ATCC 15233	22.5	11	4.19	2.85
	32.5		3.55	2.78
Candida albicans NCPF 3179	22.5	39	2.27	2.17
	32.5		1.89	1.28
Candida famata 300502	22.5	12	2.25	2.15
	32.5		4.74	4.02
Penicillium chrysogenum ATCC 9179	22.5	10	6.14	6.14
	32.5		8.58	8.33
Bacteria				
Bacillus subtilis NCTC 10400	22.5	16	1.10	1.12
	32.5		0.55	0.55
Burkholderia cepacia NCTC 10743	22.5	30	1.71	1.80
	32.5		0.75	0.76
Penicillium chrysogenum ATCC 9179	22.5	15	2.35	2.46
	32.5		1.13	1.17
Corynebacterium striatum ATCC BAA-1293	22.5	19	4.64	5.81
	32.5		1.23	1.40
Enterococcus faecalis NCTC 12697	22.5	22	1.48	1.52
	32.5		0.68	0.71
Escherichia coli NCTC 12923	22.5	35	1.19	1.34
	32.5		0.63	0.63
Kocuria rhizophila NCTC 8340	22.5	23	3.22	2.81
	32.5		1.65	1.36
Kocuria varians 10085	22.5	29	3.45	3.47
	32.5		2.05	1.91
Methylobacterium extorquens NBRC 15911	22.5	17	5.84	6.22
	32.5		4.96	6.44
Micrococcus luteus ATCC 11880	22.5	36	5.10	2.47
	32.5		2.84	1.28
Pseudomonas aeruginosa NCTC 12924	22.5	30	1.88	1.95
	32.5		0.91	0.94
Ralstonia pickettii 109228	22.5	16	3.25	3.46
	32.5		1.32	1.53
Staphylococcus aureus NCTC 10788	22.5	34	1.95	2.06
	32.5		0.85	0.85
Staphylococcus epidermidis NCTC 6513	22.5	19	4.78	4.46
	32.5		1.10	1.05
Staphylococcus hominis 8045	22.5	38	5.53	4.50
	32.5		1.19	1.12
Staphylococcus warneri 12953	22.5	10	4.18	4.34
	32.5		1.01	1.02
Staphylococcus warneri 12953	22.5	5	2.53	2.81
	32.5		1.13	1.21
Stenotrophomonas maltophilia ATCC 13637	22.5	16	1.67	1.82
	32.5		0.71	0.76
Streptomyces albidoflavus ATCC 25422	22.5	29	9.83	8.59
	32.5		2.95	3.64
Yersinia enterocolitica ATCC 9610	22.5	14	2.19	1.87
	32.5		1.02	0.97

Neutralization: Neutralization results in the iFA Plus and adjusted iFA Plus formulations are presented in Table 3. Comparison results of the adjusted iFA Plus and BACTEC Plus Aerobic/F neutralization capacity are presented in Table 4. All seeded bottle types tested without antimicrobials were positive at all 3 temperatures (data not shown). For a given antimicrobial/microorganism pair, seeded non-neutralizing (iAST/BACTEC Aerobic Standard) bottles were tested to confirm antimicrobial efficacy and were negative (data not shown).

RESULTS AND DISCUSSION (CONTINUED)

Table 3. Time-to-detection (in days) of iFA Plus and adjusted iFA Plus in the Presence of Antimicrobials

Microorganism	Antimicrobial & Concentration (ug/bottle)	Incubation	Median TTD (Days)	
			iFA Plus	Adjusted iFA Plus
B. subtilis NCTC 10400	Amikacin; 300	22.5	1.75	1.80
		32.5	0.75	0.83
B. subtilis NCTC 10400	Streptomycin; 500	22.5	2.74	2.66
		32.5	1.29	1.87
C. albicans NCPF 3179	Amphotericin B; 10	22.5	2.18	2.16
		32.5	2.02	1.34
S. aureus NCTC 1078	Amikacin; 300	32.5	0.80	0.80

The adjusted iFA Plus formulation tested in the presence of an antimicrobial agent recovered (100%) the susceptible microorganism evaluated within the expected recovery time of no more than three (3) days for bacteria and not more than five (5) days for yeast .

Table 4. Recovery and TTD in adjusted iFA Plus and BACTEC Plus Aerobic/F in the Presence of Antimicrobials.

Organism	Antimicrobial Agent	Penase1 or Not?	Adjusted iFA Plus			BACTEC Plus Aerobic/F		
			Incubation Temp.	Average TTD (Days)	# Positive/ Tested	Incubation Temp.	Average TTD (Days)	# Positive/ Tested
A. brasiliensis NCPF 2275	Amphotericin B	None	22.5°C	3.26	5/5	-	-	-
			32.5°C	1.75	5/5	35.0°C	1.91	5/5
	Cocktail	Penase	22.5°C	3.62	5/5	-	-	-
			32.5°C	1.68	5/5	35.0°C	1.95	5/5
B. subtilis NCTC 10400	Amikacin	None	22.5°C	1.70	5/5	-	-	-
			32.5°C	0.76	5/5	35.0°C	0.88	1/5
	Cocktail	Penase	22.5°C	2.09	5/5	-	-	-
			32.5°C	1.48	5/5	35.0°C	0.59	5/5
	Penicillin G	None[2]	22.5°C	1.68	5/5	-	-	-
			32.5°C	0.82	5/5	35.0°C	NG	0/5
		Penase	22.5°C	1.57	5/5	-	-	-
			32.5°C	0.70	5/5	35.0°C	0.48	5/5
	Streptomycin	None	22.5°C	2.20	5/5	-	-	-
			32.5°C	1.24	5/5	35.0°C	0.57	5/5
	Vancomycin	None	22.5°C	1.63	5/5	-	-	-
			32.5°C	0.68	5/5	35.0°C	0.62	5/5
C. albicans NCPF 3179	Amphotericin B	None	22.5°C	1.93	5/5	-	-	-
			32.5°C	1.13	5/5	35.0°C	2.21	5/5
	Cocktail	Penase	22.5°C	1.83	5/5	-	-	-
			32.5°C	1.15	5/5	35.0°C	2.28	5/5
S. aureus NCTC 10788	Amikacin	None	22.5°C	2.02	5/5	-	-	-
			32.5°C	0.78	5/5	35.0°C	NG	0/5
	Cocktail	Penase	22.5°C	1.98	5/5	-	-	-
			32.5°C	0.82	5/5	35.0°C	0.97	5/5
	Penicillin G	None2	22.5°C	3.07	5/5	-	-	-
			32.5°C	1.00	5/5	35.0°C	NG	0/5
		Penase	22.5°C	2.10	5/5	-	-	-
			32.5°C	0.84	5/5	35.0°C	0.79	5/5
	Streptomycin	None	22.5°C	2.18	5/5	-	-	-
			32.5°C	0.94	5/5	35.0°C	2.38	2/5
	Vancomycin	None	22.5°C	2.06	5/5	-	-	-
			32.5°C	0.77	5/5	35.0°C	1.38	5/5

[1] **Penase:** Pen G at 500 U per bottle, alone or in cocktail
[2] **NO** Penase: Pen G at 24 (*B. subtilis*) or 50 U (*S. aureus*) per bottle
NG: No growth

- All 160/160 (100%) adjusted BTA iFA Plus bottles tested with microorganisms paired with antimicrobials were declared positive.
- Only 58/80 (72.5%) BACTEC Plus Aerobic/F bottles were able to support growth in the presence of antimicrobial agents.
- The BACTEC Plus Aerobic/F bottles failed to support growth of *B. subtilis or S. aureus* in the presence of 24 U Penicillin G. This test was performed without adding Penase, meaning that the antibiotic activity was intact, and demonstrates that the BACTEC bottles are unable to reduce the concentration of Penicillin G to a level lower than MIC of the microorganisms.
- The BACTEC bottles did not support growth of *S. aureus* in the presence of Amikacin.
- Only 1/5 (20%) BACTEC Plus Aerobic/F bottles recovered *B. subtilis* in the presence of Amikacin and only 2/5 (40%) partially recovered *S. aureus* paired with Streptomycin.

CONCLUSION

The adjusted BTA iFA PLUS medium has no negative impact on the recovery and detection of the ICH and industrial microorganisms at incubation temperatures used by industry customers in both the presence and absence of antimicrobials.

In the presence of antimicrobials, 100% (160/160) of the adjusted iFA Plus bottles were able to support growth of all microorganisms at both 22.5°C and 32.5°C. Only 58/80 (72.5%) BACTEC Plus Aerobic/F bottles were able to support growth in the presence of antimicrobial agents.

In the presence of antimicrobials, the Adjusted iFA Plus bottle had a faster TTD for *A. brasiliensis* and *C. albicans* at 32.5°C than BACTEC Plus Aerobic/F bottle tested at 35°C.

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