OPTIMIZING AUTOMATED GROWTH-BASED METHODS FOR FASTER STERILITY RELEASE TESTING OF CELL & GENE THERAPY PRODUCTS

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INTRODUCTION

Automated Growth-based methods are used for rapid detection of microbial contamination in pharmaceutical products such as Cell and gene therapy (CGT). For therapies on the critical path to patient treatment, every day/hour gained on release testing is crucial to accelerate delivery to patients in need.

The BACT/ ALERT® 3D (BTA) Solution provides an automated non-destructive growth-based rapid microbial method and is considered a reference method by both European Pharmacopeia (EP) chapter 2.6.27 and United States (USP) chapter <72>. Although most relevant microorganisms are detected in less than 72h, the current practice is the release of product after 7 days incubation, mainly due to the risk of contamination with 'slow growing' microorganisms such as molds, *Cutibacterium acnes* or *Micrococcus luteus.* To improve the detection of such microorganisms, different culture media, growth factors and incubation conditions were evaluated. Applying USP <72> guidelines, the minimum incubation time was determined and compared to current practices..

PURPOSE OF THIS WORK

Present data demonstrating how optimizing incubation conditions and supplementing BACT/ALERT® media can significantly improve time to detection, in alignment with the new USP <72> chapter, thereby reducing detection of microbial contamination timelines and accelerating CGT product release.

MATERIALS AND METHODS

BACT/ALERT® 3D DUAL-T

- Growth/Respiration based method for detection of aerobic and anaerobic bacteria and fungi using combination of aerobic & anaerobic industry bottle as required by Pharmacopeias [1-2].
- Determine presence of microorganisms based on dissolved CO_2 production continuous monitoring thanks to a colorimetric sensor and reflected light measurement.

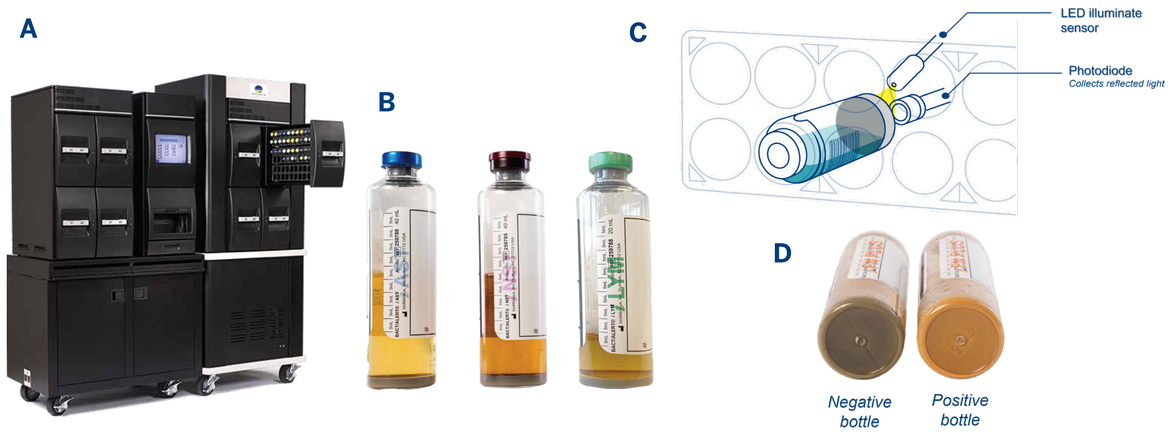

Figure 1. A/ BACT/ALERT® 3D DUAL-T system; B/ Industry culture media bottle; C/ Detection principle; D/ Colorimetric sensor indicative of positive result.

STUDY OUTLINE

- 11 microbial strains were inoculated into BACT/ALERT® Industry standard bottles (iAST for aerobic testing, or iLYM for aerobic testing optimized for yeast and molds, iNST for anaerobic testing) or supplemented bottles without product (Table 1). Un-inoculated bottles were included as negative controls.
- When applicable, supplementation consisted of addition of growth factor solutions[5-8] prepared in-house (Table 2) or commercially available ready-to-use supplement (BD BBL™ IsoVitaleX™) promoting growth of fastidious organisms.
- A minimum of 3 bottles were seeded at low concentration ≤ 10 CFU per bottle, with inoculum prepared from a fresh culture or a BIOBALL® and incubated at either 22°C, 32°C or 36°C.
- Mean Time to Detection (MTTD) data for each microorganism was compared between the various conditions tested.
- The "safety margin" was calculated following USP <72> guidelines by using growth curve enumeration on plates to determine the generation time of the slowest-growing microorganism.

Table 1: List of microorganisms and incubation conditions evaluated.

Microorganism	Collection strain (BIOBALL or *fresh culture)	Initial Incubation conditions	Optimal Incubation conditions
Aspergillus brasiliensis	NCPF 2275	iAST 22.5°C	iLYM 22.5°C
Bacillus spizizenii	NCTC 10400	iAST 22.5°C	iAST 32.5°C
Candida albicans	NCPF 3179		
Micrococcus luteus	Isolate*		
Pseudomonas paraaeruginosa	NCTC 12924		
Streptococcus pyogenes	ATCC 19615*		
Bacteroides fragilis	ATCC 25285*	iNST 32.5°C	iNST 36°C or iNST 36°C + commercial supplement
Clostridium sporogenes	NCTC 12935		
Cutibacterium acnes	DSM 1897		
Phocaeicola vulgatus (formerly *Bacteroides vulgatus)*	ATCC 8482*		
Staphylococcus aureus	NCTC 10788		

Table 2: List of growth factors evaluated against commercially available supplement.

Growth factors	Reference	Concentration of stock solution	Gain in MTTD in hours at 36°C
Bovine Serum Albumin (BSA) Solution 30%	Sigma-Aldrich A9576	10 % (v/v)	-0.90
Iron (III) nitrate nonahydrate	Sigma-Aldrich F8508	0,025 g/L	0.27
L-Cysteine hydrochloride mononhydrate	Sigma-Aldrich C7880	50 g/L	0.59
L-Cystine	Sigma-Aldrich C8755	1 g/L	0.42
L-Glutathione Reduced	Sigma-Aldrich G6013	50 g/L	0.52
N-Acetyl-L-cysteine	Sigma-Aldrich A9165	50 g/L	0.79
Nicotinamide-adénine-dinucléotide (NAD) hydrate	Sigma-Aldrich N7004	0.25 g/L	0.1
Tween 80	Sigma-Aldrich W291706	20% (v/v)	-0.04
BD BBL™ IsoVitaleX™	BD	-	0.78

RESULTS

- All negative controls were confirmed negative after 7 days by the system & visual inspection (data not shown). All inoculum were ≤ 10 CFU with the exception of B. fragilis & P. vulgatus respectively at 21 & 12 CFU and *C. acnes* in iNST + L-Cysteine at 12 CFU.
- Figure 2 & 3 display respectively baseline & optimized performances of aerobic and anaerobic strains. Table 2 presents gain in *C. acnes* MTTD when using iNST bottle supplemented with various growth factors or commercial supplement compared to non-supplemented media.

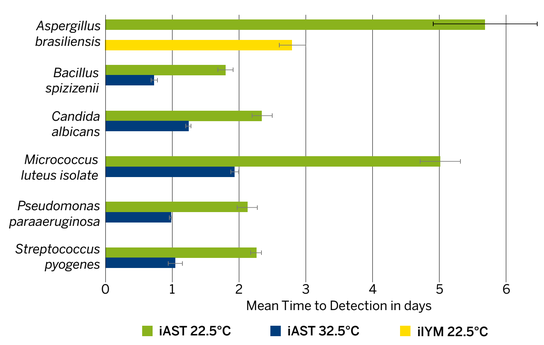

Figure 2: Mean time to detection in days of aerobic microorganisms using aerobic bottle iAST 22.5°C, iAST 32.5°C & fungal-specific aerobic bottle iLYM 22.5°C (n = 10).

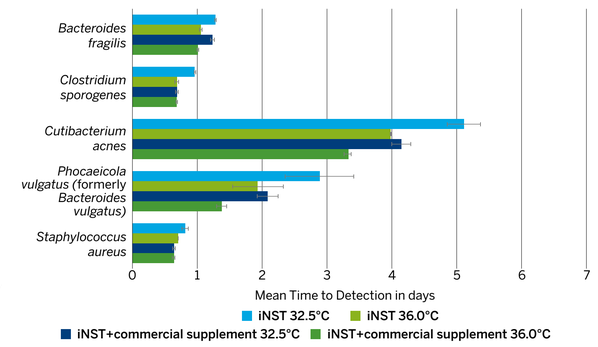

Figure 3: Mean time to detection in days of anaerobic microorganisms using iNST 32.5°C, iNST 36°C, iNST + commercial supplement at 32.5°C and iNST + commercial supplement at 36°C (n = 3 to 5 except for C. sporogenes, C. acnes & S. aureus in iNST 32.5°C where n = 10).

C. acnes growth curves based on enumeration on agar plates were generated for all 4 conditions (data not shown) starting with a fresh culture and used to determine the generation time, the safety margin & the incubation time to be applied according to USP <72> (Table 3).

Table 3: Calculation of the incubation time as per USP <72> in the different incubation conditions for C. acnes

Growth conditions in absence of product	Mean time to detection in days	Mean generation time in hours	Safety margin calculation in days	Incubation time in the product to be examined in days
iNST 32°C	5.11	3.00	0.41	5.52
iNST 36°C	3.99	2.70	0.37	4.36
iNST + supplement 32°C	4.15	2.50	0.34	4.49
iNST + supplement 36°C	3.34	2.30	0.32	3.66

DISCUSSION

- Using a combination of iAST 22.5°C & iNST 32.5°C bottle, all microbial strains inoculated ≤ 10 CFU in absence of product, were detected within 7 days (Figures 2 & 3).
- Addition of a 3rd aerobic bottle at 32.5°C helped reduce MTTD of some aerobic bacteria such as *M. luteus* (< 3 days instead of 5) (Figure 2).
- As described by Daane & Jeffrey[3], MTTD of molds can be further optimized using iLYM bottle: *A. brasiliensis* was recovered at 22.5°C in less than 3 days (Figure 2). Incubation at low temperature is critical to recover other environmental isolates including *Aspergillus* spp. or *Penicillium* spp. (3)
- *C. acnes* exhibits maximum growth at human body temperatures. Incubation at 36°C demonstrated consistent gain in MTTD of about 1 day for *C. acnes* (Figure 3) and was also beneficial on *B. fragilis* & *P. vulgatus* (Fig 3).
- Additional growth factors described in the literature[4-7] were also screened and compared against commercially available supplement (Table 2). Some ingredients such as Iron nitrate (0.25 g/L), NAD (0.25 g/L) had limited effect on *C. acnes* growth. On the contrary, BSA (10% v/v) showed a detrimental effect[8]. Contrary to what was reported by Kumaran & Ramirez-Arcos[7], addition of Tween 80 did not bring major (results were reproduced, data not shown). Sulfur-containing amino acids contributed positively to a faster growth of *C. acnes*[6] and on some occasion demonstrated similar performance but no better or worst performances when compared to commercial supplement.
- The commercial supplement was also evaluated on all anaerobic strains (Figure 3). Positive or neutral impact was observed on MTTD leading to a combined improvement in MTTD of nearly 1 days at 32.5°C and nearly 2 days at 36°C.
- Finally, the determination of *C. acnes* generation time (Table 3) - still the slowest among the tested microorganisms - supported that increased temperature and supplementation improve *C. acnes* growth, thereby optimizing the safe release timeline for CGT products. Applying the safety margin as per USP <72>, we show that the incubation time can be significantly improved and reduced from 7 days to less than 4 days based on our data in absence of product.

CONCLUSION

The automated growth-based BACT/ALERT® 3D DUAL-T system remains the gold standard for sterility testing of CGT products. In the context of USP <72>, the data presented provide an opportunity to optimize using (iLYM 22.5°C, iAST 32.5°C & iNST+supplement 36°C), and thus significantly decrease the time to release CGT products, in particular for the slowest growing organism, *C. acnes.* Applying USP <72> guidelines, our data in absence of product shows that current practice with 7 days incubation time could be amended to safely release CGT products 3 days earlier allowing therapies to reach patient more quickly.

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