Product Critical Quality Attributes: Opportunities and Challenges for Early Phase Clinical Trials

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Points to Consider

Opportunity

- Comparability testing of Critical Quality Attributes (CQA) for global collaborations

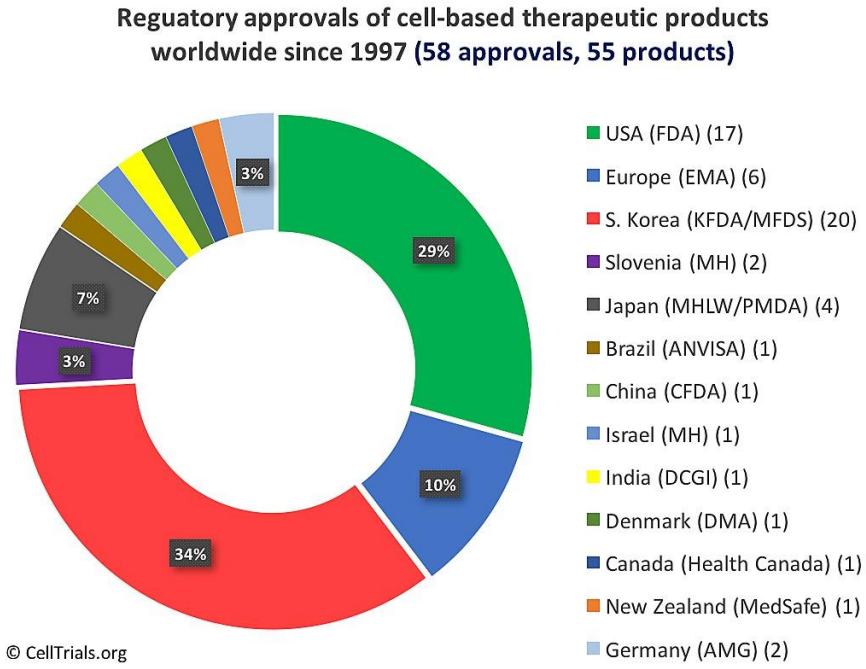
Challenge

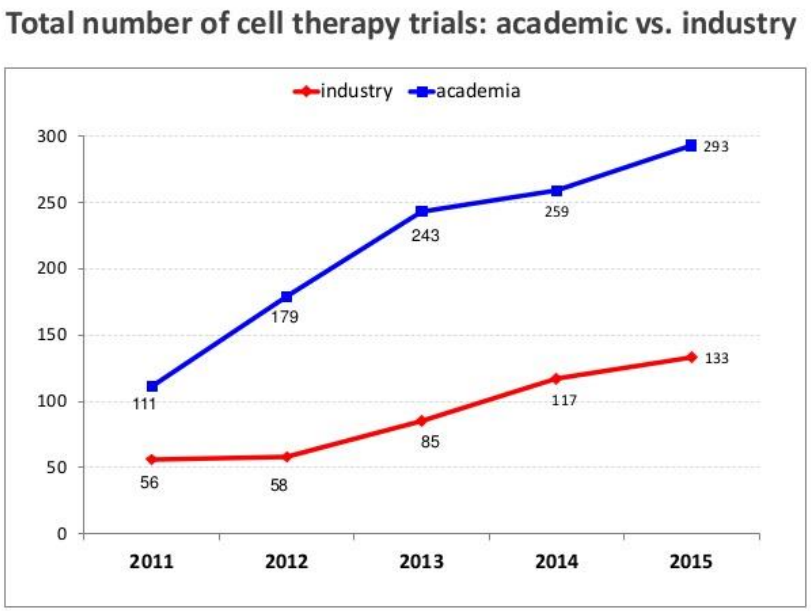
- Implementing timely and cost-effective CQA rapid release assays

Opportunity

Comparability testing of Critical Quality Attributes (CQA) for global collaborations

Leveraging Partnerships

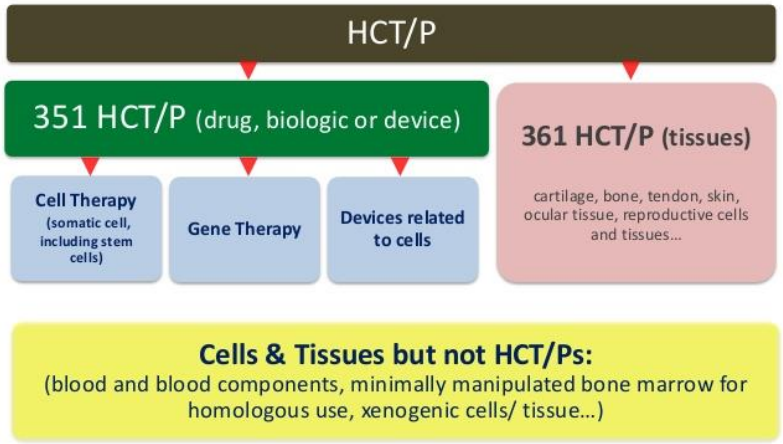




Bersenev, Alexey (2017):
DOI: [10.6084/m9.figshare.4829182](https://doi.org/10.6084/m9.figshare.4829182)

Navigating Regulations

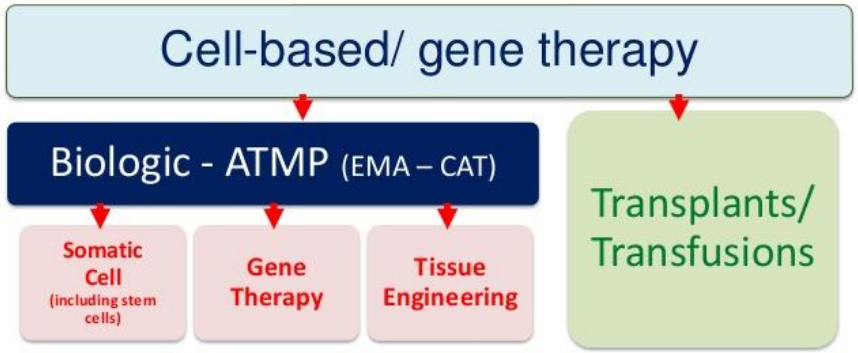
US



Food Drug Administration (FDA)

United States Pharmacopeia (USP)

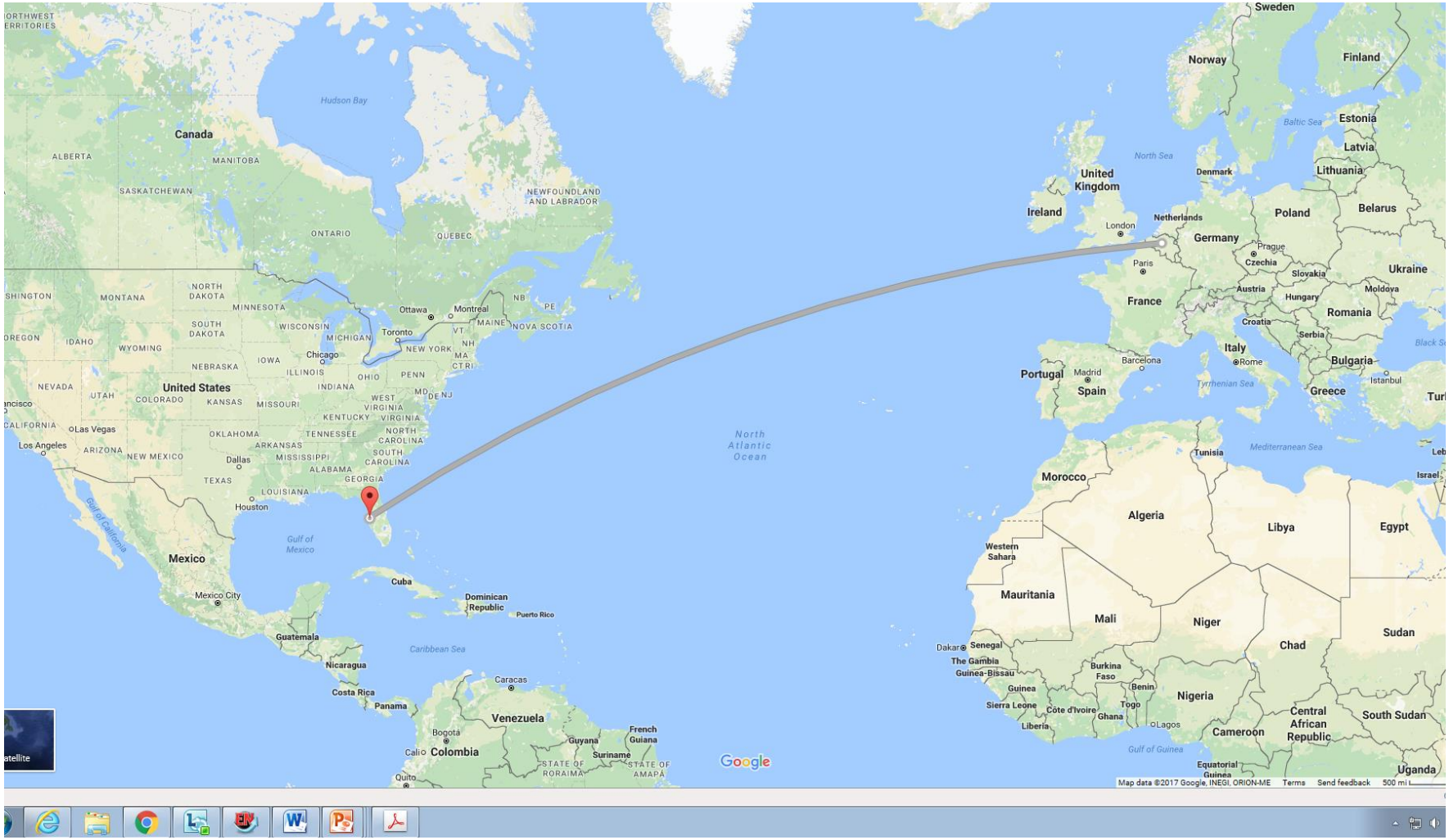
EU



European Medicines Agency (EMA)

European Pharmacopeia (EU)

International Multi-Center Phase I/II Clinical Trial
Two Manufacturing Sites



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Process Overview

Critical Quality Attributes Testing

WHEN

- Technology transfer
- Product development
- Process changes

- Do this before tackling the manufacturing protocol!

HOW

- Validated analytical methods
- Well developed SOPs
- Known reference samples
- Identical critical reagents
- Established acceptance criteria
- See one, partake one, do one
- Document in Share File

Critical Quality Attributes

Criteria	Methods	Limits
Viability	Flow cytometry	≥ 70%
Purity (CD3+ cells)	Flow cytometry	≥ 80%
Cell count	Flow cytometry	Specified dose ± 25%
Identity (NKG2D+ cells)	Flow cytometry	≥ 50%
Microbiological tests		
Sterility	Ph. Eur. 2.6.27	No growth
Endotoxin	Ph. Eur. 2.6.14	≤ 8.67 EU/mL
Mycoplasma	Ph. Eur. 2.6.7	No mycoplasma
Safety tests		
Vector copy number	Ph. Eur. 2.6.21	< 5.0 copies/cell
Replication Competent Virus	Ph. Eur. 2.6.21	No detection

Challenge

Implementing timely and cost-effective CQA rapid release assays

Microbiological Sterility Testing

FDA Draft Guidance for Industry

Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products

Published February 11, 2008
Withdrawn May 8, 2015

Introduction of iFA Plus and iFN Plus Neutralizing Media for BacT/ALERT® 3D Systems

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INTRODUCTION

Companies testing samples with antimicrobial properties require a neutralization test method to ensure detection of contaminants. Samples tested using the sterility reference method (filtration) require at least three rinses with neutralizing diluent prior to testing. Samples that cannot be filtered must be diluted to mitigate inhibition. New neutralizing media for detection of aerobic (iFA Plus) and anaerobic (iFN Plus) microorganisms were developed for BacT/ALERT 3D (BTA) Systems. The new media require no pretreatment of samples.

Antibiotics are often used in biologics and vaccines cell culture media and biopsy and tissue transport media to mitigate potential contaminants. This media is routinely tested to monitor in-process samples from bioreactors and cellular therapy manufacturing. Some commonly used antibiotics include aminoglycosides (gentamicin, streptomycin, neomycin, amikacin), beta-lactams (Penicillin G) and the antifungal Amphotericin B.

A study was performed on the BTA Dual-T system to demonstrate growth promotion and neutralizing properties of the new media. Growth promotion was demonstrated using 25 microbial genera important to industry applications. Neutralization was demonstrated through recovery of microorganisms in the presence of antibiotics at levels representing industry use. Preliminary testing using Pharmacopoeia recommended bacteria showed *B. subtilis* and *S. aureus*, respectively, to be sensitive indicators of the neutralization of antibiotics in the iFA Plus and iFN Plus media. The Pharmacopoeia recommended fungal isolates, *A. brasiliensis* and *C. albicans*, were used to demonstrate the neutralization of Amphotericin B

MATERIALS AND METHODS

A minimum of ten bottle replicates and five tubed media replicates per microorganism were seeded with inocula prepared from culture or from BTF BioBall™ to target levels of <100 cfu / bottle or tube. A hemacytometer was used to standardize mold inocula. Aerobic iFA Plus bottles were incubated at 22.5°C and 32.5°C. Anaerobic iFN Plus bottles were incubated at 32.5°C. Broth tubes (FTM and/or TSB) were incubated according to the harmonized compendia.

Growth Promotion: Mean Time to Detection (TTD) data for each microorganism/medium/temperature combination were compared to the first day that growth was observed in the direct inoculation reference method.

Neutralization: Mean TTD data for each microorganism/medium/antibiotic combination tested were compared to the mean TTD of positive controls. Concentrated stock solutions of each drug were prepared according to USP <81> or CLSI and injected into the BTA media using ≤1 ml to minimize any dilution effect. Non-neutralizing BTA media (iAST and iNST) were used as negative controls. Negative test bottles were examined microscopically and subcultured to confirm absence of growth.

RESULTS AND DISCUSSION

Results listed in Table 1 describe performance of a variety of microorganisms in the iFA Plus and iFN Plus media. For some strains of fungi, >100 cfu / bottle was tested. Tests were not repeated since the inocula were observed to be pure.

The BTA iFA Plus bottle (incubated at 32.5C or 22.5°C), and the BTA iFN Plus bottle (32.5°C) provides a suitable environment for a variety of aerobic, anaerobic and facultative bacteria, and fungi. *B. cereus* and *M. extorquens* showed less than 100% recovery in iFN Plus and iFA Plus (22.5°C), respectively. Although *B. cereus* is considered a facultative spore former, growth preference is in an aerobic medium. Additionally, *M. extorquens* is a temperature sensitive strain and performed better at an incubation temperature closer to the optimal growth temperature of 28°C.

For all strains tested, 32 of 35 have mean TTD results within 1 day of or faster than the reference method regardless of bottle type and temperature tested.

M. extorquens, P. expansum, and *P. acnes* had mean TTD results greater than the reference method. All are considered slow growing microorganisms and are recoverable in the BTA media.

Table 1. Growth Performance for iFA Plus and iFN Plus media (Days)

Microorganism	cfu	iFA Plus		iFN Plus		TSB / FTM (Days)
		Incubation Temperature (°C)	Mean TTD (Days)	Incubation Temperature (°C)	Mean TTD (Days)	
Aerobic and Facultative Bacteria						
Acinetobacter baumannii	11	22.5 32.5	1.5 0.7			2
Bacillus cereus	3	22.5 32.5	1.4 0.6	32.5	0.9*	1
Bacillus subtilis	28	22.5 32.5	1.8 0.7			1
Brevundimonas diminuta	43	22.5 32.5	2.7 1.4			2
Burkholderia cepacia	18	22.5 32.5	2.5 1.1			2
Enterobacter cloacae	27	22.5 32.5	1.3 0.7	32.5	0.7	1
Escherichia coli	25	32.5	0.6	32.5	0.6	1
Klebsiella oxytoca	25	22.5 32.5	1.3 0.7	32.5	0.7	1
Klebsiella pneumoniae	16	22.5 32.5	1.3 0.6	32.5	0.6	1
Kocuria rhizophila	13	22.5 32.5	3.6 1.7			6
Pseudomonas aeruginosa	30	22.5 32.5	2.1 0.9			2
Sphingomonas paucimobilis	60	22.5 32.5	2.8 1.4			1
Staphylococcus aureus	24	32.5	0.9	32.5	1.1	1
Staphylococcus hominis	18	32.5	1.5	32.5	2.2	3
Staphylococcus lugdunensis	22	32.5	1.3	32.5	1.3	2
Streptococcus agalactiae	23	32.5	1	32.5	1.2	1
Streptococcus mutans	29	32.5	1.8	32.5	1.9	2
Streptococcus pyogenes	16	32.5	0.7	32.5	1.6	1
Methylobacterium extroquens	36	22.5 32.5	7.0* 4.9			3
Micrococcus luteus	7	22.5 32.5	9.3 2.6			6
Yersinia enterocolitica	13	32.5	1.1	32.5	1.2	1
Yeast and Mold						
Aspergillus brasiliensis	34	22.5 32.5	4.0 2.0			4
Aspergillus niger	8	22.5 32.5	4.1 2.2			2
Candida albicans	29	22.5 32.5	2.7 2.7			3
Candida parapsilosis	71	22.5 32.5	2.7 1.5			5
Cryptococcus neoformans	44	22.5 32.5	6.3 4.8			12
Cladosporium cladosporioides	26	22.5	3.2			3
Fusarium solani	300	22.5 32.5	3.5 2.5			2
Penicillium chrysogenum	265	22.5	3.6			3
Penicillium expansum	217	22.5	3.3			2
Anaerobic Bacteria						
Actinomyces bovis	69			32.5	3.3	4
Bacteroides fragilis	62			32.5	3.4	3
Bacteroides vulgatus	38			32.5	2.1*	3
Clostridium sporogenes	60			32.5	1.2	2
Propionibacterium acnes	22			32.5	8.3	5

*Indicates less than 100% recovery
+ Performance results with 0.5mL lysed horse blood

Results in Table 2 show performance in the presence of common antimicrobial agents. The levels chosen represent those encountered in industrial settings.

Amphotericin B, Penicillin G, and Streptomycin are neutralized in the iFA Plus and iFN Plus media. All indicator strains tested display mean TTDs equivalent to or faster than the control bottle (no antimicrobials added).

Table 2. Antimicrobial Neutralization iFA Plus and iFN Plus

Microorganism**	Drug (Concentration)	iFA Plus		Control	iFN Plus		Control
		Incubation Temperature (°C)	Mean TTD (Days)	iFA Plus Mean TTD (Days)	Incubation Temperature (°C)	Mean TTD (Days)	iFN Plus Mean TTD (Days)
Aspergillus brasiliensis	Amphotericin B (10 mcg)	22.5 32.5	3.9 2.0	3.5 2.1			
Candida albicans	Amphotericin B (10 mcg)	22.5 32.5	2.5 2.3	2.4 2.2			
Bacillus subtilis	Penicillin G (500 IU) + 0.1mL Penase	22.5 32.5	1.6 0.7	1.5 0.7			
	Penicillin G (24 IU)	22.5 32.5	1.7 0.8	1.5 0.7			
	Streptomycin (500 mcg)	22.5 32.5	2.3 1.0*	1.5 0.7			
Staphylococcus aureus	Penicillin G (500 IU) + 0.1mL Penase	32.5	1.0	0.9	32.5	0.8	1.0
	Penicillin G (50 IU)	32.5	1.1	0.8	32.5	1.2	1.1
	Streptomycin (1000 mcg)				32.5	1.1	1.0

* Indicates less than 100% recovery
** Less than 100 cfu per bottle were inoculated

The highest level of Penicillin G neutralized in the iFA Plus medium is 24 IU and 50 IU for *B. subtilis* and *S. aureus*, respectively. In the presence of concentrated Penase, detection is observed when ten times or more of Penicillin G is introduced into either bottle type.

For Streptomycin and *Bacillus subtilis*, 2 of 120 replicates were negative for growth. No assignable cause was attributed and subsequent testing did not reproduce the result. Stasis testing confirmed that the resin could neutralize the antimicrobial to allow for growth of the microorganism.

CONCLUSION

- **The BacT/ALERT iFA Plus and iFN Plus media provide growth promoting and neutralization properties that are comparable to the reference method for a variety of bacteria, yeast, and fungi and several classes of antimicrobials. Testing is via direct inoculation..**
- **The BacT/ALERT 3D Dual-T system used with the new iFA Plus and iFN Plus neutralizing media is a fully automated, non-destructive, alternative microbiological method that requires no pretreatment and minimal sample manipulation for testing samples containing antimicrobials.**

REFERENCES

1. EP 2.6.1, Sterility
2. JP 4.06, Sterility Test
3. USP <71>, Sterility Tests
4. USP <81>, Antimicrobial Effectiveness Testing
5. USP <1227>, Validation of Microbial Recovery from Pharmaceutical Articles
6. CLSI M27-A3, Reference Method for Broth Dilution Antifungal Susceptibility Testing

Note: The iFA Plus and iFN Plus media are not yet commercially available.

Replication Competent Retroviral (RCR) Testing

Guidance for Industry

Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research (CBER)
October 2000

Replication-Competent Retroviruses in Gene-Modified T Cells Used in Clinical Trials: Is It Time to Revise the Testing Requirements?

Adham S Bear, Richard A Morgan, Kenneth Cornetta, Carl H June, Gwendolyn Binder-Scholl, Mark E Dudley, Steven A Feldman, Steven A Rosenberg, Sheila A Shurtleff, Cliona M Rooney, Helen E Heslop and Gianpietro Dotti

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