

Stability

Christopher A Bravery

cbravery@advbiols.com

1



Consulting on Advanced Biologicals

CBMP – Do these Stability Data Look Suitable?

Real-time stability 4 °C; Batch #1234.

Attribute	Test method	Specification	Time-point		
			0	1	2
Appearance	Visual inspection	red/orange turbid solution	complies	complies	complies
Identity	Phenotypic marker (flow cytometry)	complies	complies	complies	complies
Viability	Trypan blue (manual count)	≥ 70%	95	94	92
Potency	Bioassay	≥ 4 U/10 ⁶ cells	12.1	9.8	6.8
Sterility	EP/USP	No growth	No growth	NT	No growth

Necessary but don't evaluate stability?

Is this assay sensitive to change?

Is this taking up too much of the DP?

Overall WEAK specification

Too few time-points

What does this really tell you?

- Audience participation
- identify any issues with these stability data, test methods and testing schedule.

2



Consulting on Advanced Biologicals

CBMP – Better?

Attribute	Test method	Specification	Time-point		
			0	1	2
Appearance	Visual inspection	red/orange turbid solution	complies	complies	complies
Identity	Phenotypic marker (flow cytometry)	complies	complies	complies	complies
Viability	Trypan blue (manual count)	≥ 70%	95	94	92
Content	Manual cell count	≥ 10 ⁶ cells/mL	2.1 × 10 ⁶	1.8 × 10 ⁶	1.1 × 10 ⁶
Potency	Bioassay	≥ 4 U/10 ⁶ cells	12.1	9.8	6.8
Sterility	EPI/USP	No growth	No growth	NT	No growth

Now viability can be interpreted

Viability fairly stable

Nearly 50% of cells lost

- Presenting viability as a percentage without total cells is meaningless.
 - *Yet I see this often?*

3



Consulting on Advanced Biologicals

CBMP – Different ways to present data

Attribute	Test method	Specification	Time-point		
			0	1	2
Appearance	Visual inspection	red/orange turbid solution	complies	complies	complies
Identity	Phenotypic marker (flow cytometry)	complies	complies	complies	complies
Viability	Trypan blue (manual count)	≥ 70%	95	94	92
Non-viable cells	Trypan blue (manual count)	≤ 0.3 × 10 ⁶ non-viable cells/mL	0.1 × 10 ⁶	0.1 × 10 ⁶	0.1 × 10 ⁶
Total Content	Manual cell count	≥ 10 ⁶ cells/mL	2.1 × 10 ⁶	1.8 × 10 ⁶	1.1 × 10 ⁶
Content	Manual cell count	≥ 10 ⁵ viable cells/mL	2.0 × 10 ⁵	1.7 × 10 ⁵	1.0 × 10 ⁵
Potency	Bioassay	≥ 4 U/10 ⁶ cells	12.1	9.8	6.8
Sterility	EPI/USP	No growth	No growth	NT	No growth

Product-related Impurity

Total Content

Could be useful to include purity (specific cell content) in terms of viable cell content.

- Note: this is not a recommendation, merely to make the point that you need to consider the best way to present data.

4



Consulting on Advanced Biologicals

Take-Home Message

What Are You Trying to Measure?

- **Measurement:** process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity.
- **Analyte:** specific substance to be measured (e.g. IL-2, haemoglobin).
- **Measurand:** quantity to be measured

12 Focus – Advanced therapies

Measurement reliability over the cellular therapeutic product lifecycle

See discussion in these

Cytotherapy 16(9): 1187-1196.

International Society for Cellular Therapy
ISCT

Reference materials for cellular therapeutics

CHRISTOPHER A. BRAVERY¹ & ANNA FRENCH²

¹Consulting on Advanced Biologicals Ltd, Advanced Biologicals Ltd, London, United Kingdom, and ²The Oxford-UCL Centre for the Advancement of Sustainable Medical Innovation (CASMI), The University of Oxford, Oxford, United Kingdom

Abstract
The development of cellular therapeutics (CTP) takes place over many years, and, where successful, the developer will anticipate the product to be in clinical use for decades. Successful demonstration of manufacturing and quality consistency is

Author
Christopher A Bravery, Consulting on Advanced Biologicals Ltd, London, UK.

What are reference materials?
In metrology all measurements are made with respect to a reference. For example the amount of protein in a sample will be defined in mg units per unit volume (mg/ml) only if the development of that unit is a common task, yet how often have you stopped to question why you are applying and, importantly, why using a particular reference material as the test method?

Regulatory Rapporteur – Vol 12, No 5, May 2015

CAB

Biotech Example

	Attribute	Test method	Specification	Time-point (months)							
				0	3	6	9	12	18	24	30
General Tests	Appearance	Visual inspection	Colourless to yellowish solution	x	x	NT	NT	x	NT	x	x
	Clarity	Visual inspection	Clear to ≤ reference suspension	x	x	NT	NT	x	NT	x	x
	pH	Potentiometry	4.2 – 4.8	x	x	x	x	x	x	x	x
	Extractable volume	Weight	≤ 0.5 ml	x	x	NT	NT	x	NT	x	x
Identity	Molecular size	SEC	Corresponds to reference	x	x	x	x	x	x	x	x
	Hydrophobicity	RP-HPLC	Corresponds to reference	x	x	x	x	x	x	x	x
	Isoelectric point	IEF	Corresponds to reference	x	NT	x	NT	x	x	x	x
Purity	High MW variants	SEC	Σ High MW variants ≤ 0.2%	x	x	x	x	x	x	x	x
	Product related impurities	RP-HPLC	Σ ≤ 6.5% Largest individual impurity ≤ 3.0%	x	x	x	x	x	x	x	x
	Charged variants	IEF	≤ 7 minor bands 2 – 5% 0 minor bands > 5%	x	NT	x	NT	x	x	x	x
	Bacterial endotoxins	Ph. Eur. 2.6.14	≤ 5 IU/ml	x	NT	NT	NT	NT	NT	x	x
	Particulate matter	Ph. Eur. 2.9.19	< 6000 ≥10 µm/syringe < 800 ≥25 µm/syringe	x	NT	NT	NT	x	NT	x	x
	Foreign matter	Ph. Eur. 2.9.20	Practically free from particles	x	x	x	x	x	x	x	x
	Sterility	Ph. Eur. 2.6.1	No growth	x	NT	NT	NT	x	NT	NT	x
Content Assay	RP-HPLC	0.54 – 0.63 mg/ml (90 – 105%)	x	x	x	x	x	x	x	x	

Biotech Example: Release v End of Shelf-life

Attribute	Test method	Specification	Time-point (months)								
			0	3	6	9	12	18	24	30	
General Tests	Appearance	Visual inspection	Colourless to yellowish solution	x	x	NT	NT	x	NT	x	x
	Clarity	Visual inspection	Clear to ≤ reference suspension	x	x	NT	NT	x	NT	x	x
	pH	Potentiometry	4.2 – 4.8	x	x	x	x	x	x	x	x
	Extractable volume	Weight	≤ 0.5 ml	x	x	NT	NT	x	NT	x	x
Identity	Molecular size	SEC	Corresponds to reference	x	x	x	x	x	x	x	x
	Hydrophobicity	RP-HPLC	Corresponds to reference	x	x	x	x	x	x	x	x
	Isoelectric point	IEF	Corresponds to reference	x	NT	x	NT	x	x	x	x
Purity	High MW variants	SEC	Σ High MW variants ≤ 5%	x	x	x	x	x	x	x	x
	Product related impurities	RP-HPLC	Σ ≤ 6.5% Largest individual impurity ≤ 3.0%	x	x	x	x	x	x	x	x
	Charged variants	IEF	≤ 7 minor bands 2 – 5% 0 minor bands > 5%	x	NT	x	NT	x	x	x	x
	Bacterial endotoxins	Ph. Eur. 2.6.14	≤ 5 IU/ml	x	NT	NT	NT	NT	NT	x	x
	Particulate matter	Ph. Eur. 2.9.19	< 6000 ≥10 µm/syringe < 600 ≥25 µm/syringe	x	x	NT	NT	NT	x	NT	x
	Foreign matter	Ph. Eur. 2.9.20	Practically free from particulate matter	x	x	x	x	x	x	x	x
	Sterility	Ph. Eur. 2.6.1	No growth	x	x	x	x	x	x	NT	x
	Content Assay	RP-HPLC	0.54 – 0.63 mg/ml (90 – 105%)	x	x	x	x	x	x	x	x

Release Specification
Sum ≤ 4.0%
Largest individual impurity ≤ 2.0%

Release Specification
≤ 3 minor bands 2 – 5%
0 minor bands > 5%

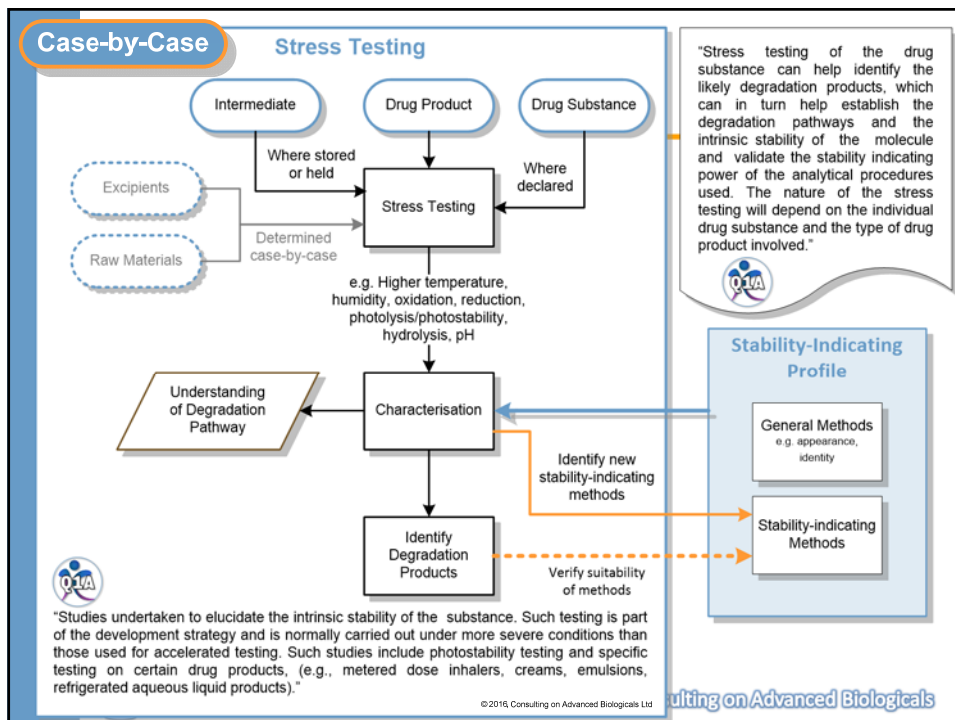
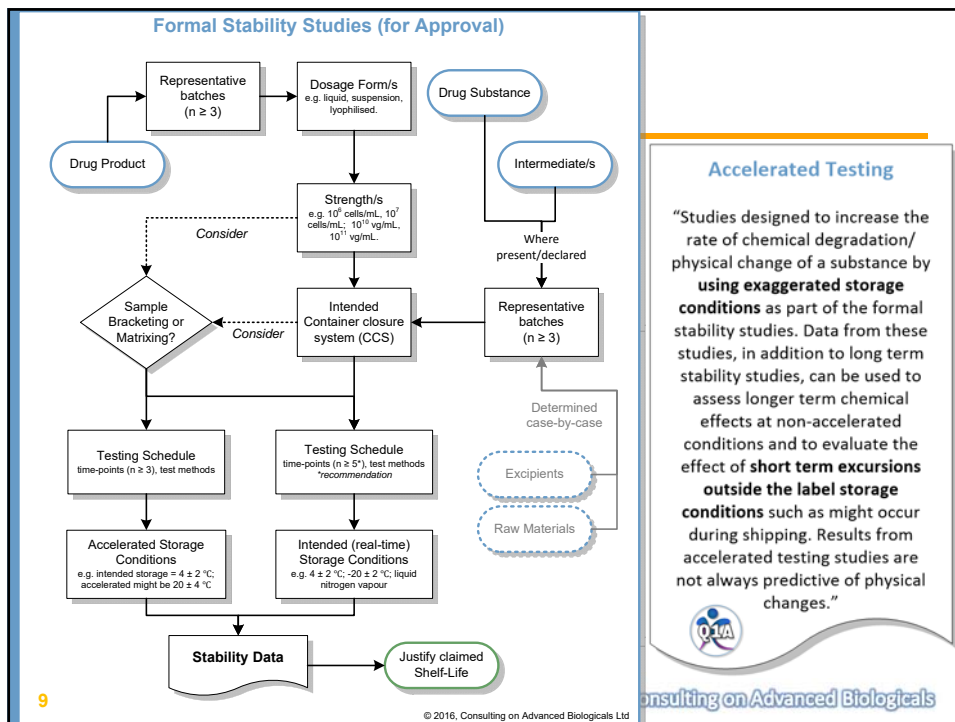
ICH - General Principles

Specifics = case-by-case



<http://advbiols.com/documents/ICH.swf>

Consulting on Advanced Biologicals



Identifying Stability-Indicating Parameters

Considerations-1

- Unlike (inert) proteins, cells are not in equilibrium with their environment (i.e. viable/alive/living).
 - Resistant to oxidation/reduction etc
 - protective mechanisms, repair mechanisms etc
 - How to identify changes leading up to (sudden?) loss of cell integrity/viability?
- Common viability methods (e.g. trypan blue) give only yes/no result for viability of each cell
 - explore earlier changes preceding loss of viability
 - e.g. apoptosis, autophagy, necrosis
 - Classification of cell death: *Cell Death Differ.* 2009 January ; 16(1): 3–11. doi:10.1038/cdd.2008.150.
 - Molecular definitions: *Cell Death and Differentiation* (2012) 19, 107–120

11



Consulting on Advanced Biologicals

Identifying Stability-Indicating Parameters

Considerations-2

- Measures of cell stress, e.g.
 - Biochemical
 - Physical (membrane integrity)
 - Secreted factors, e.g. heat-shock proteins etc.
- Receptor/ligand modulation
 - Surface receptor/ligands involved in the MoA may be induced, up/down-regulated, internalised/shed.
 - mRNA changes normally precede protein changes by hours to days.

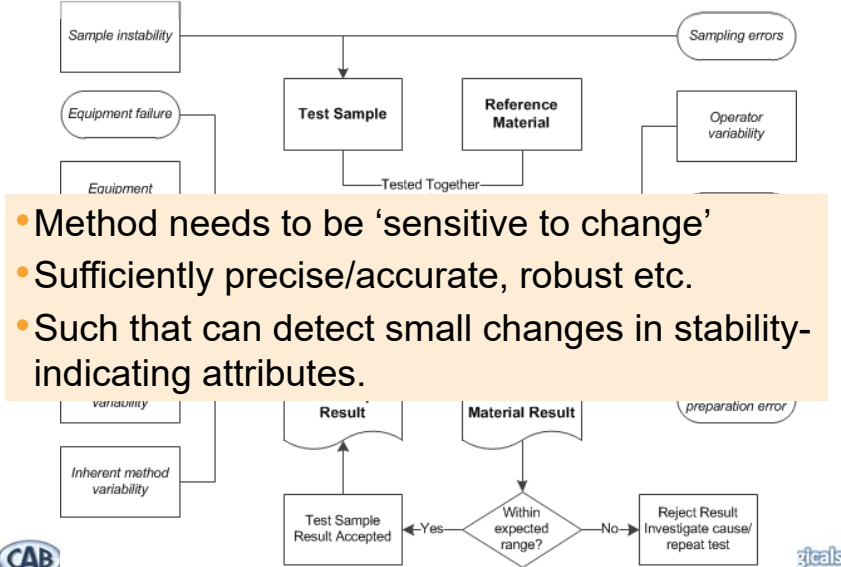
12



Consulting on Advanced Biologicals

Sources of Analytical Error

Bravery & French; Cytotherapy 16(9): 1187-1196.



- Method needs to be 'sensitive to change'
- Sufficiently precise/accurate, robust etc.
- Such that can detect small changes in stability-indicating attributes.

Other Stability Studies

(Not discussed in this talk)

- In-use stability
 - Mimic situation at clinical site
- Shipping
 - Shipping studies (real ± simulated) to evaluate impact on stability
- Raw materials
 - Where stability uncertain
 - Buffers, media, and other solutions prepared on-site
- QC samples
 - Duration of storage and handling before testing
 - Shipping to external tests labs

Other Uses for Stability-Indicating Methods

- Extended characterisation
- Comparability Studies

15



Consulting on Advanced Biologicals

Conclusions 1/2

Before a stability specification and tests protocol can be devised;

- Need to understand the stability-indicating profile (including stability-indicating parameters)
 - General characterisation
 - Stress-testing
- Need to identify stability-indicating methods (to measure stability-indicating parameters)
 - Sensitive to change
 - Sufficiently precise, (accurate), robust etc.
- These data will be needed to set and justify your stability specification and acceptance criteria.

16



Consulting on Advanced Biologicals

Conclusions 2/2

- Overall testing can be reduced in some instances by the use of Bracketing and/or Matrixing
 - Warning: use appropriately
- Release and Stability specifications and acceptance criteria can differ
 - Not all release tests are stability-indicating
 - e.g. process-related impurities unlikely to change
 - It may be necessary to accept some parameters change gradually over the shelf-life;
 - Stability (end of shelf-life) > Release
 - Needs justification, e.g. toxicity data (safe levels)
 - e.g. viability for fresh cell products.

17



Consulting on Advanced Biologicals

But I haven't had Questions about Stability? So they must think its OK....??

Stage	Thorough & Appropriate Development	Quality	Safety	Efficacy
Clinical Development (CTA/IND etc)	x	✓ as relates to safety	✓	x/✓
Approval (MAA/BLA etc)	✓	✓	✓	✓

- Clinical trials assessment/review does **not** evaluate whether you are on-track with development.
- Main assessment criterion is **safety of the trial subjects**.
- It's **the developers responsibility** to ensure overall development is on-track and meets current expectations and standards.

18



Consulting on Advanced Biologicals