Stability

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CBMP – Do these Stability Data Look Suitable?

Real-time s… 4 ℃; Batch #1234.

Overall WEAK specification

Too few time-points

Attribute	Test method	Specifiction	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?

What does this really tell you?

Is this assay sensitive to change?

Is this taking up too much of the DP?

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

CBMP – Better?

Attribute	Test method	Specifiction	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	EP/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?*

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CBMP – Different ways to present data

Attribute	Test method	Specifiction	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
~~Content~~	~~Manual cell count~~	~~≥ 10^6 cells/mL~~	~~2.1 × 10^6~~	~~1.8 × 10^6~~	~~1.1 × 10^6~~
Content	Manual cell count	≥ 10^6 viable cells/mL	2.0 × 10^6	1.7 × 10^6	1.0 × 10^6
Potency	Bioassay	≥ 4 U/10^6 cells	12.1	9.8	6.8
Sterility	EP/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.**

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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

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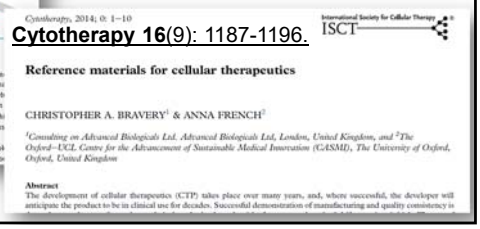
Regulatory Rapporteur – Vol 12, No 5, May 2015

See discussion in these


Cytotherapy, 2014; 0: 1–10

Cytotherapy 16(9): 1187-1196.

ISCT

Reference materials for cellular therapeutics

CHRISTOPHER A. BRAVERY[1] & ANNA FRENCH[2]

Abstract

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Biotech Example

	Attribute	Test method	Specifiction	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 < 600 ≥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

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Biotech Example: Release v End of Shelf-life

	Attribute	Test method	Specifiction	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
Identity	Molecular size	SEC	Corresponds to referer							x	x
	Hydrophobicity	RP-HPLC	Corresponds to referer							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 ≤	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		NT	NT	NT	x	NT	x	x
	Foreign matter	Ph. Eur. 2.9.20	Practically free from partic							x	x
	Sterility	Ph. Eur. 2.6.1	No growth							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%

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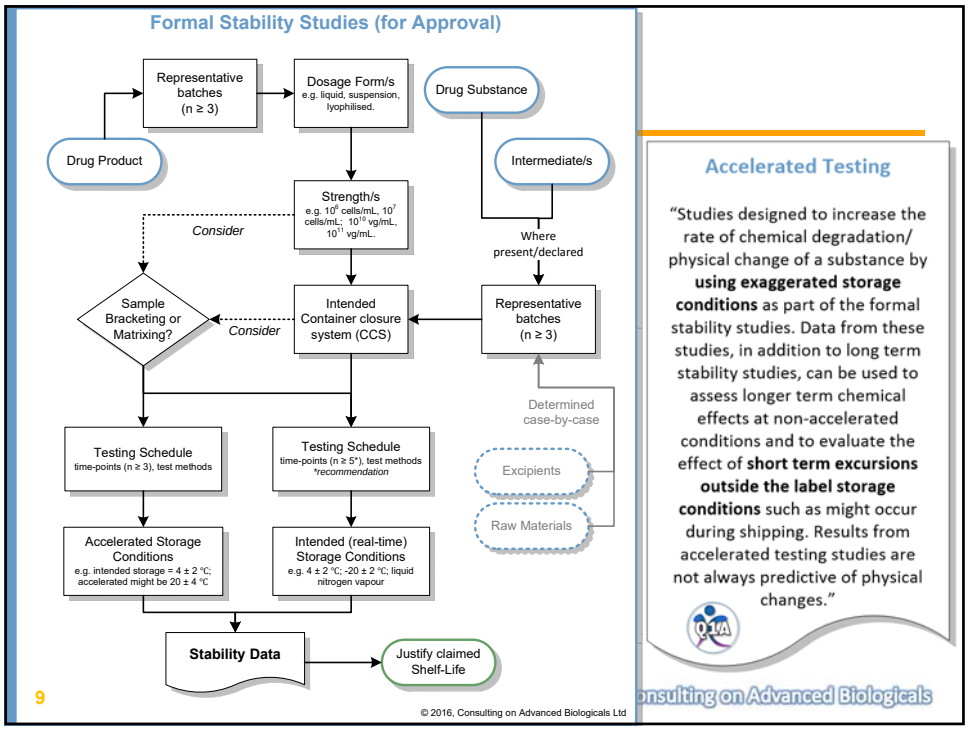
ICH - General Principles

Specifics = case-by-case

CAB http://advbiols.com/documents/ICH.swf Consulting on Advanced Biologicals


Formal Stability Studies (for Approval)
Representative batches (n ≥ 3)
Dosage Form/s
e.g. liquid, suspension, lyophilised.
Drug Substance
Drug Product
Intermediate/s
Strength/s
e.g. 10^6 cells/mL, 10^7 cells/mL; 10^10 vg/mL, 10^11 vg/mL.
Consider
Where present/declared
Sample Bracketing or Matrixing?
Consider
Intended Container closure system (CCS)
Representative batches (n ≥ 3)
Determined case-by-case
Excipients
Raw Materials
Testing Schedule
time-points (n ≥ 3), test methods
Testing Schedule
time-points (n ≥ 5*), test methods
*recommendation
Accelerated Storage Conditions
e.g. intended storage = 4 ± 2 °C; accelerated might be 20 ± 4 °C
Intended (real-time) Storage Conditions
e.g. 4 ± 2 °C; -20 ± 2 °C; liquid nitrogen vapour
Stability Data
Justify claimed Shelf-Life
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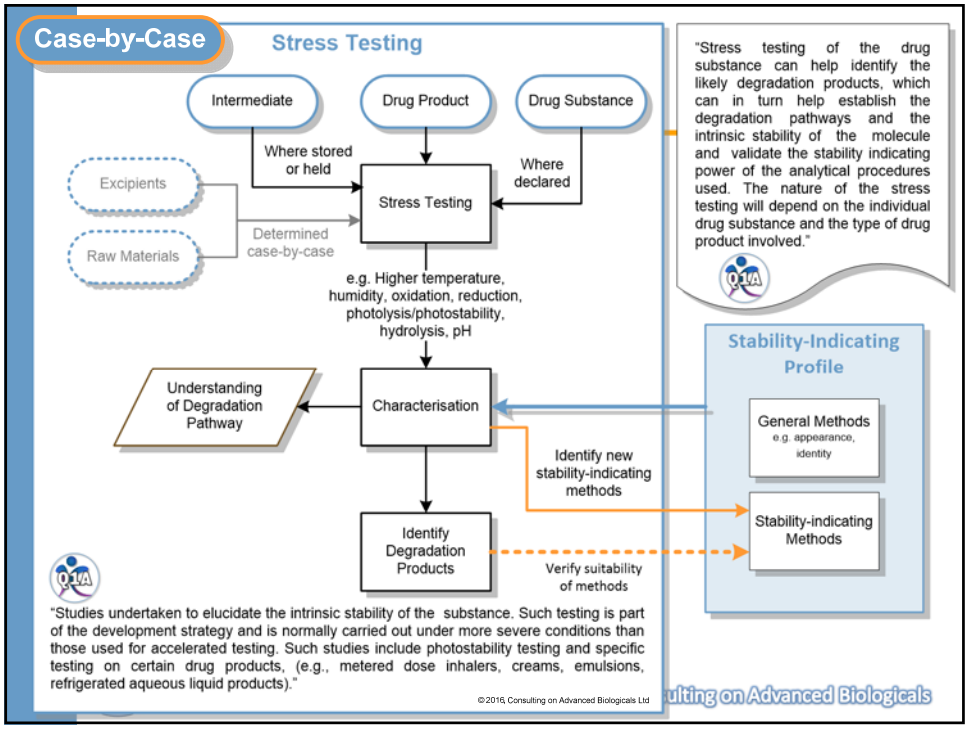
Accelerated Testing
"Studies designed to increase the rate of chemical degradation/ physical change of a substance by using exaggerated storage conditions as part of the formal stability studies. Data from these studies, in addition to long term stability studies, can be used to assess longer term chemical effects at non-accelerated conditions and to evaluate the effect of short term excursions outside the label storage conditions such as might occur during shipping. Results from accelerated testing studies are not always predictive of physical changes."
onsulting on Advanced Biologicals


Case-by-Case
Stress Testing
Intermediate
Drug Product
Drug Substance
Where stored or held
Where declared
Excipients
Raw Materials
Determined case-by-case
Stress Testing
e.g. Higher temperature, humidity, oxidation, reduction, photolysis/photostability, hydrolysis, pH
Understanding of Degradation Pathway
Characterisation
Identify new stability-indicating methods
Identify Degradation Products
Verify suitability of methods
"Studies undertaken to elucidate the intrinsic stability of the substance. Such testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated testing. Such studies include photostability testing and specific testing on certain drug products, (e.g., metered dose inhalers, creams, emulsions, refrigerated aqueous liquid products)."
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"Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved."
Stability-Indicating Profile
General Methods
e.g. appearance, identity
Stability-indicating Methods
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Identifying Stability-Indicating Parameters
Considerations-1

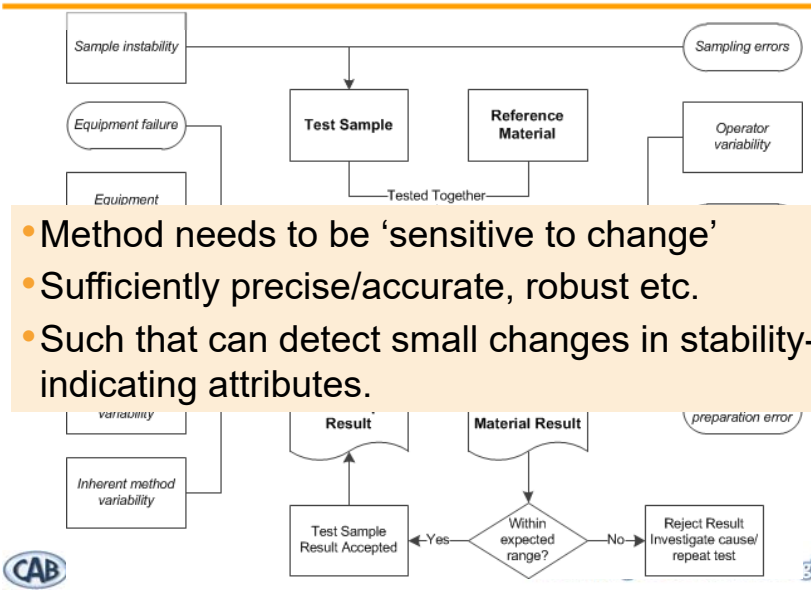
- Unlike (inert) proteins, cells are not in equilibrium with their environment (i.e. viable/alive/living).
  - Resistant to oxidation/reduction etc
    - protective mechanisms, repair mechanisms etc
  - How to identify changes leading up to (sudden?) loss of cell integrity/viability?
- Common viability 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

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.

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.

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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

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Other Uses for Stability-Indicating Methods

- Extended characterisation
- Comparability Studies

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.

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.

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)	✗	✓ as relates to safety	✓	✗/✓
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.