# Stability

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## Overview

- Issues I see with Stability Studies (cell therapy)
- How its expected to be done (ICH principles)
  - Stress testing
  - Accelerated stability
  - Real-time stability
  - In-use stability
  - Other related studies (not discussed)

## CBMP – Do these Stability Data Look Suitable?

eal-time … 4 °C; Batch #1234.

| 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?

Overall WEAK specification

Too few time-points

How can this be useful?

What does this really tell you?

Is this assay sensitive to change?

Is this taking up too much of the DP?

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## 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~~ Purity | Phenotypic marker (flow cytometry) | ~~complies~~ >80 % | ~~complies~~ 95% | ~~complies~~ 93% | ~~complies~~ 94% |
| ~~Viability~~ | ~~Trypan blue (manual count)~~ | ~~≥ 70%~~ | ~~95~~ | ~~94~~ | ~~92~~ |
| Non-viable cells | Trypan blue (manual count) | ≤ $0.3 \times 10^6$ non-viable cells/mL | $0.1 \times 10^6$ | $0.1 \times 10^6$ | $0.1 \times 10^6$ |
| ~~Content~~ | ~~Manual cell count~~ | ~~≥ $10^6$ cells/mL~~ | ~~$2.1 \times 10^6$~~ | ~~$1.8 \times 10^6$~~ | ~~$1.1 \times 10^6$~~ |
| Content | Manual cell count | ≥ $10^6$ viable cells/mL | $2.0 \times 10^6$ | $1.7 \times 10^6$ | $1.0 \times 10^6$ |
| 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**

**A measure of purity unlike identity is quantitative and likely to be stability-indicating.**

**Total cell content could be total cells or total viable cells.**

- **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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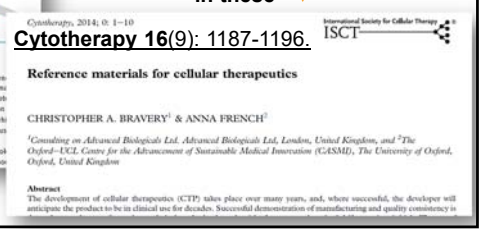
What are reference materials?

Regulatory Rapporteur – Vol 12, No 5, May 2015

**See discussion in these**


Cytotherapy, 2014; 0: 1–10

International Society for Cellular Therapy ISCT

**Cytotherapy 16**(9): 1187-1196.

Reference materials for cellular therapeutics

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

[1]Consulting on Advanced Biologicals Ltd. Advanced Biologicals Ltd, London, United Kingdom, and [2]The 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



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# ICH - General Principles

## Specifics = case-by-case



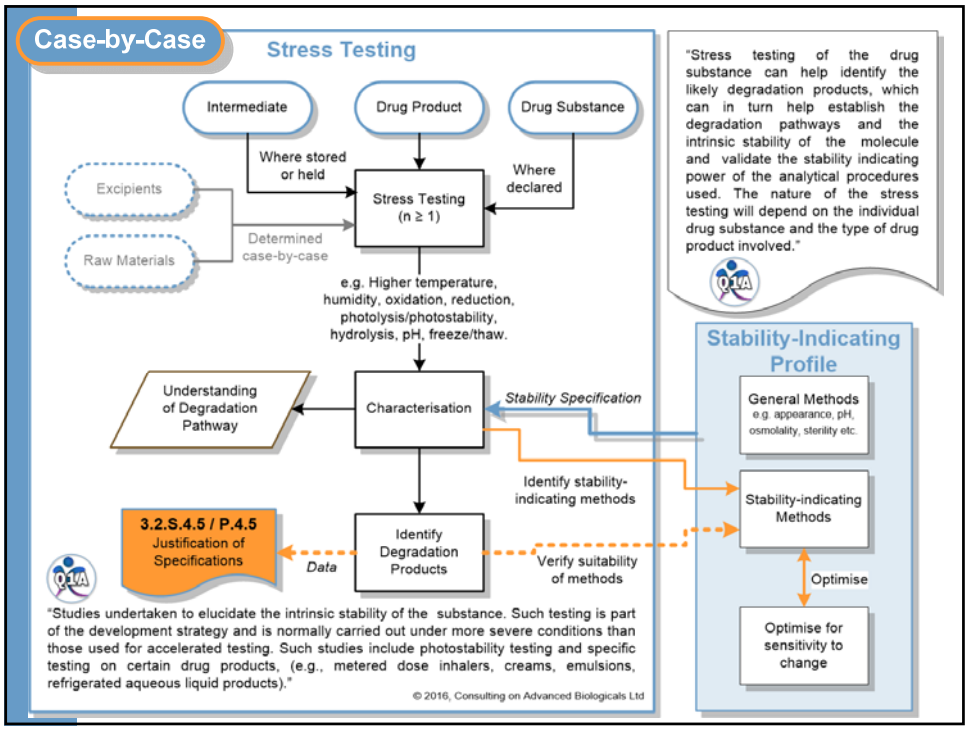

http://advbiols.com/documents/ICH.swf



## Before undertaking any stability studies, you need to determine:

- ✓ **what the *Stability-Indicating* parameters are**
- ✓ **the *most appropriate* analytical methods to evaluate these.**

- ✓ The obvious way to do this is to deliberately '*degrade*' the product (intermediate, DS etc).




# 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



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# Identifying Stability-Indicating Parameters
## Considerations-2

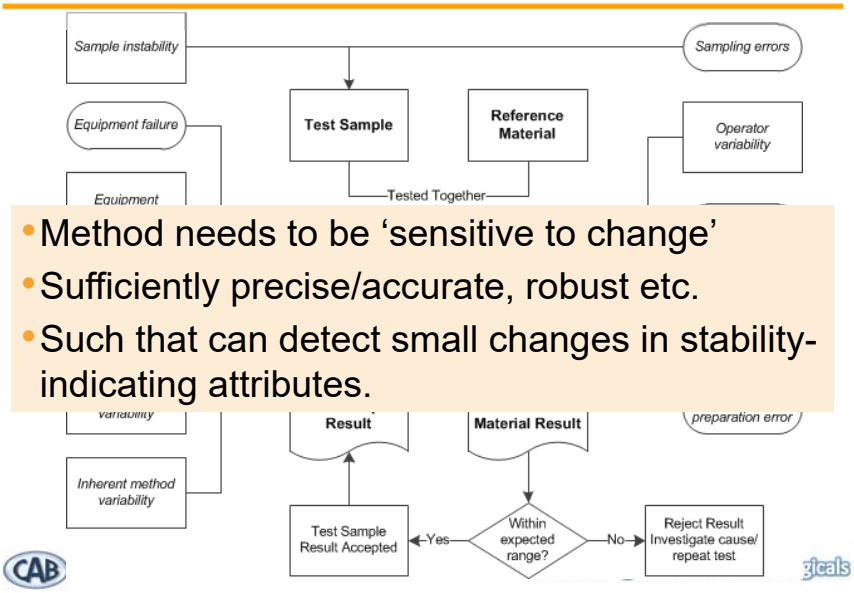
- Measures of cell stress, e.g.
  - Biochemical
  - Physical (membrane integrity)
  - Secreted factors, e.g. heat-shock proteins etc.
- Receptor/ligand modulation
  - Surface receptor/ligands involved in the MoA may be induced, up/down-regulated, internalised/shed.
    - mRNA changes normally precede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





# Sources of Analytical Error

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

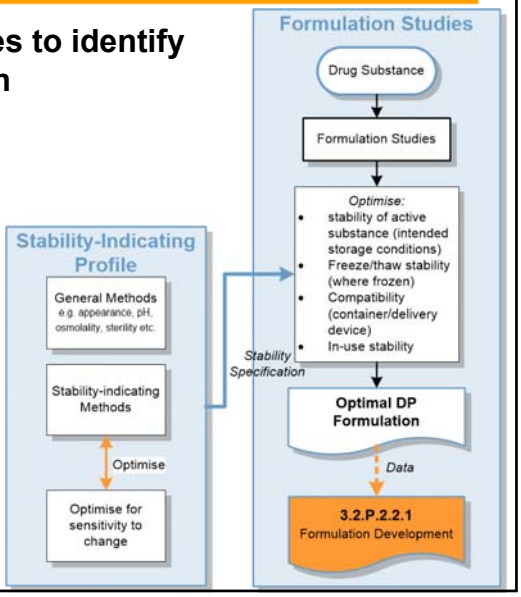



- Method needs to be 'sensitive to change'
- Sufficiently precise/accurate, robust etc.
- Such that can detect small changes in stability-indicating attributes.

# Formulation Development

**There should be studies to identify the optimal formulation**

- Issue – off-the-shelf media are not optimised for <u>your product</u> and <u>route of administration</u>
- Data support justification for excipient specifications 3.2.P.4.4.

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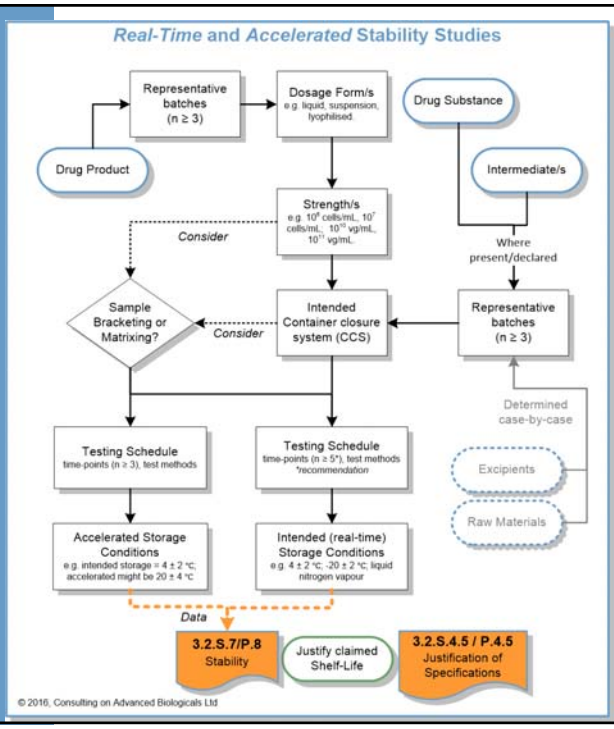
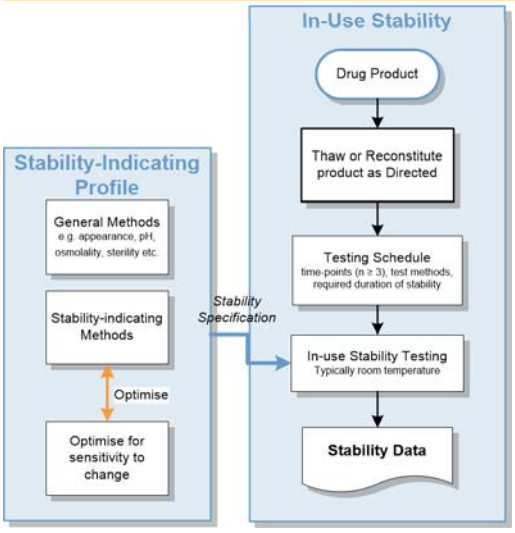



**Note:** The End of Shelf-life Specification should be in **3.2.S.4.1/P.5.1** (with Release Specification)

## 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."

## In-Use Stability (3.2.P.8)




Confirm the product is stable during clinical handling

- ✓ Thawing
- ✓ Reconstitution
- ✓ How soon
- ✓ Leave on side/put in fridge?



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## Other Stability Studies
### (Not discussed in this talk)

- 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**
  - Can be very useful because they are *sensitive to change*
    - for *extended characterisation* (i.e. beyond routine testing)
    - as well as stability studies undertaken as part of comparability.





## 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.