
Measurement reliability over the product lifecycle: *The need for reference materials*

Christopher A Bravery

cbravery@advbiols.com

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
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Overview

- Overview of measurement concepts
- What is a reference material/standard
- Measurement reference materials
- Product reference materials
- Conclusions

Cytotherapy, 2014; 16: 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

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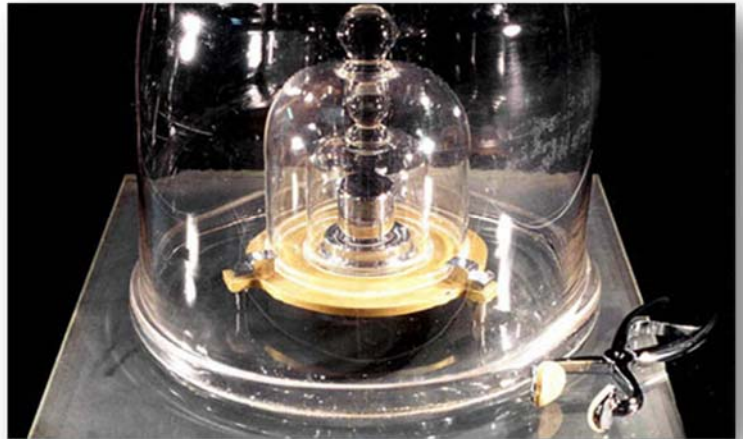


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What is a Kg?

- The international prototype of the kilogram (IPK) is the artefact whose mass defines at present the SI unit of mass: “The kilogram is the unit of mass; it is equal to the mass of the international prototype of the kilogram”.
- Held at *The International Bureau of Weights and Measures* (BIPM) near Paris, France.

The international prototype of the kilogram was sanctioned in 1889. Its form is a cylinder with diameter and height of about 39 mm. It is made of an alloy of 90 % platinum and 10 % iridium.



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What is a (physical) Standard?

- **A standard is the physical embodiment of a unit.**
- **In general a standard is not independent of physical conditions and is a true embodiment of the unit only under specified conditions.**

From: Weights and measures standards of the United States: A brief history (1963/1976) <http://www.nist.gov/pml/pubs/sp447/>

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SI Base Units

- Quantity, measuring unit (abbreviation)
- Length, metre (m)
- mass, kg (kg)
- time, second (s)
- electric current, ampere (A)
- thermodynamic temperature, kelvin (K)
- amount of substance, mole (mol)
- luminous intensity, candela (cd)

e.g. The meter is the length of the path travelled by light in vacuum during a time interval of $1/299\,792\,458$ of a second

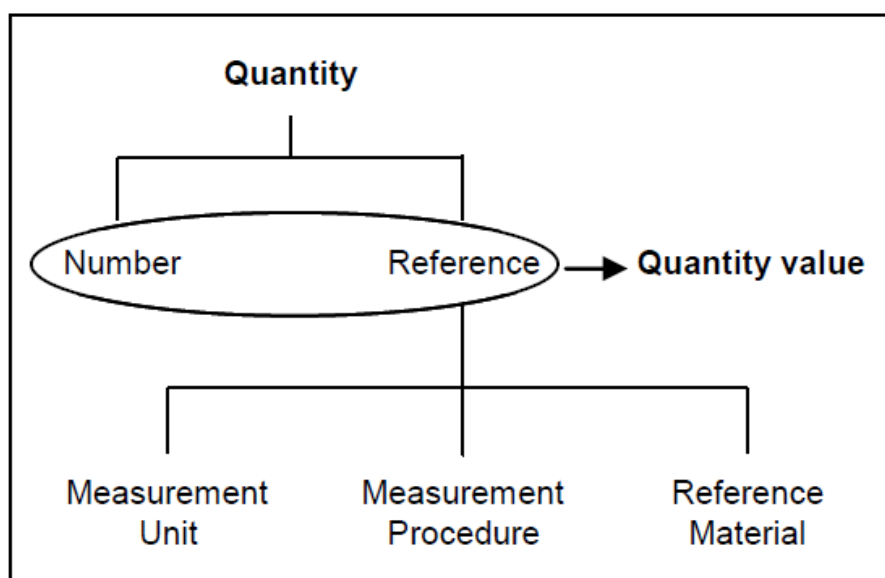
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What Are You Trying to Measure?

- **Quantity**: property of a phenomenon, body, or substance, where the property has a magnitude that can be expressed as a number and a reference.
 - i.e. something in the real world we can measure



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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

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Simple Example: Viability?

- **Analyte:** trypan blue positive cells.
 - **Measurand:** %Viability – proportion of cells that are not trypan blue positive.
 - Does *anyone* think this is useful?

 - **Analyte:** trypan blue positive.
 - **Measurand:** The number of cells not trypan blue positive in a set volume (e.g. viable cells/mL)
- or
- total viable cells (yield)
- Is this more useful?

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But measuring protein isn't simple

Table 2
Plasma protein concentration

RM=BSA

Sample (plasma), n=7	Protein concentration (graph) (mg/ml)	Protein concentration formula (BSA standard ^a) (mg/ml)
Amido Black		
Median±S.D.	83.4±1.93	82.6±1.91
CV %	2.3	2.3
Lowry		
Median±S.D.	110.2±4.09	109.1±4.31
CV %	3.7	3.9
Bradford		
Median±S.D.	95.1±3.3	89.5±3.1
CV %	3.5	3.5
Biüret		
Median±S.D.	88.8±3.54	80.9±3.67
CV %	4	4.5
Ponceau-S/TCA		
Median±S.D.	92.3±5.71	94.7±5.86
CV %	6.2	6.2

^a BSA concentration for Biüret assay 0.5 mg/ml; Lowry, Bradford, Amido Black and Ponceau-S/TCA assays were 0.1 mg/ml.

^b HSA concentration for Biüret assay 0.5 mg/ml; Lowry, Bradford, Amido Black and Ponceau-S/TCA assays were 0.05 mg/ml.

Different methods (different measurement principle) will give different results (same analyte) – this is why orthogonal methods are important as part of characterisation.

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J. Biochem. Biophys. Methods 70 (2007) 709–711

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Potency Example: Identifying RM

- **Potency assay:** secretion of cytokine X by product.
- **Analyte:** soluble cytokine 'X'.
- Measurement system: ELISA
- Certified reference material (standard) for cytokine X from NIBSC/WHO/NIST or similar.
 - ! Check purpose of RM, e.g. is it just for activity or is it for protein content?
 - If none is available you may have to prepare your own.
- The RM here is used to calibrate (as a calibrant) the assay.

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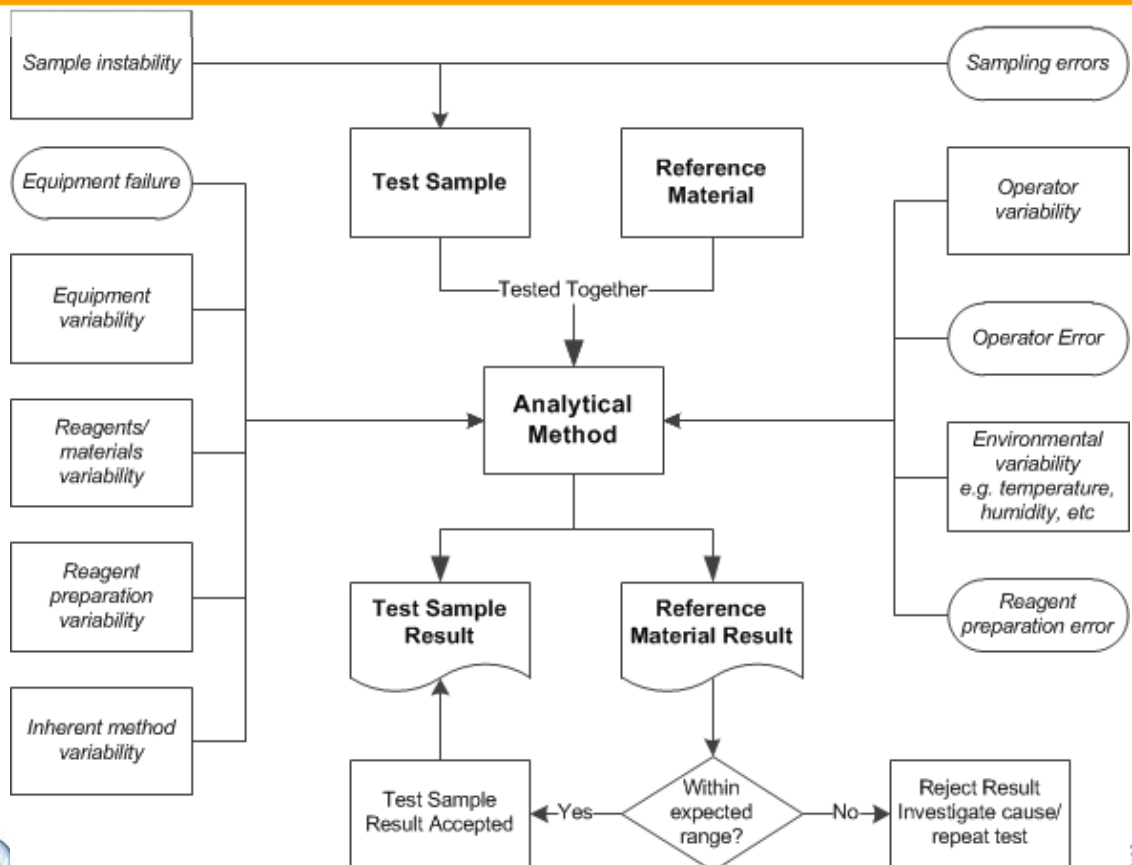
ENOUGH?

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Potency Example: Identifying RM

- **Measurand:** Mass (concentration) of 'X' secreted in response to a stimulus over 48 hours by 10^5 cells/mL in DMEM+10% FCS.
- Many things might affect this;

Why we use reference materials



Potency Example: Identifying RM

- **Measurand:** Mass (concentration) of 'X' secreted in response to a stimulus over 48 hours by 10^5 cells/mL in DMEM+10% FCS.
- Ideally here you would have a RM composed of cells that have been shown to secrete 'X' consistently in response to the stimulus in a way that is representative of the cell to be measured.
- But this is likely to be tricky; imaginative solutions may be needed,

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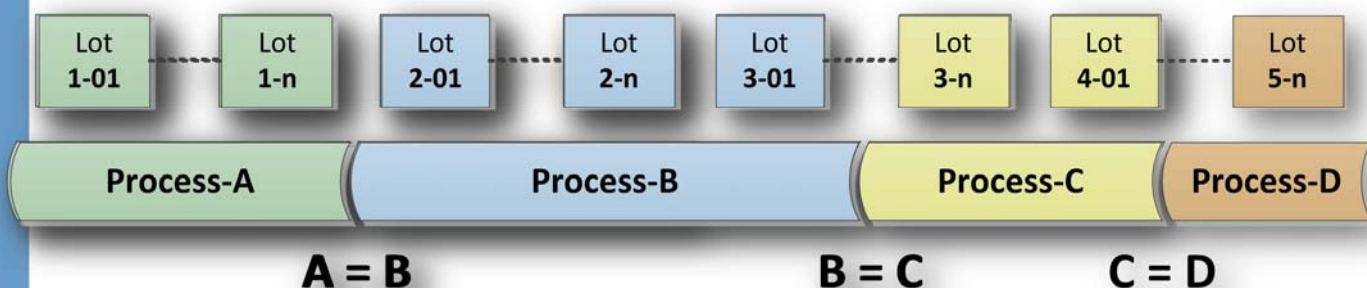


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Product Reference Materials

- Change is inevitable
 - Change in materials
 - Change in process
 - Change in analytical methods

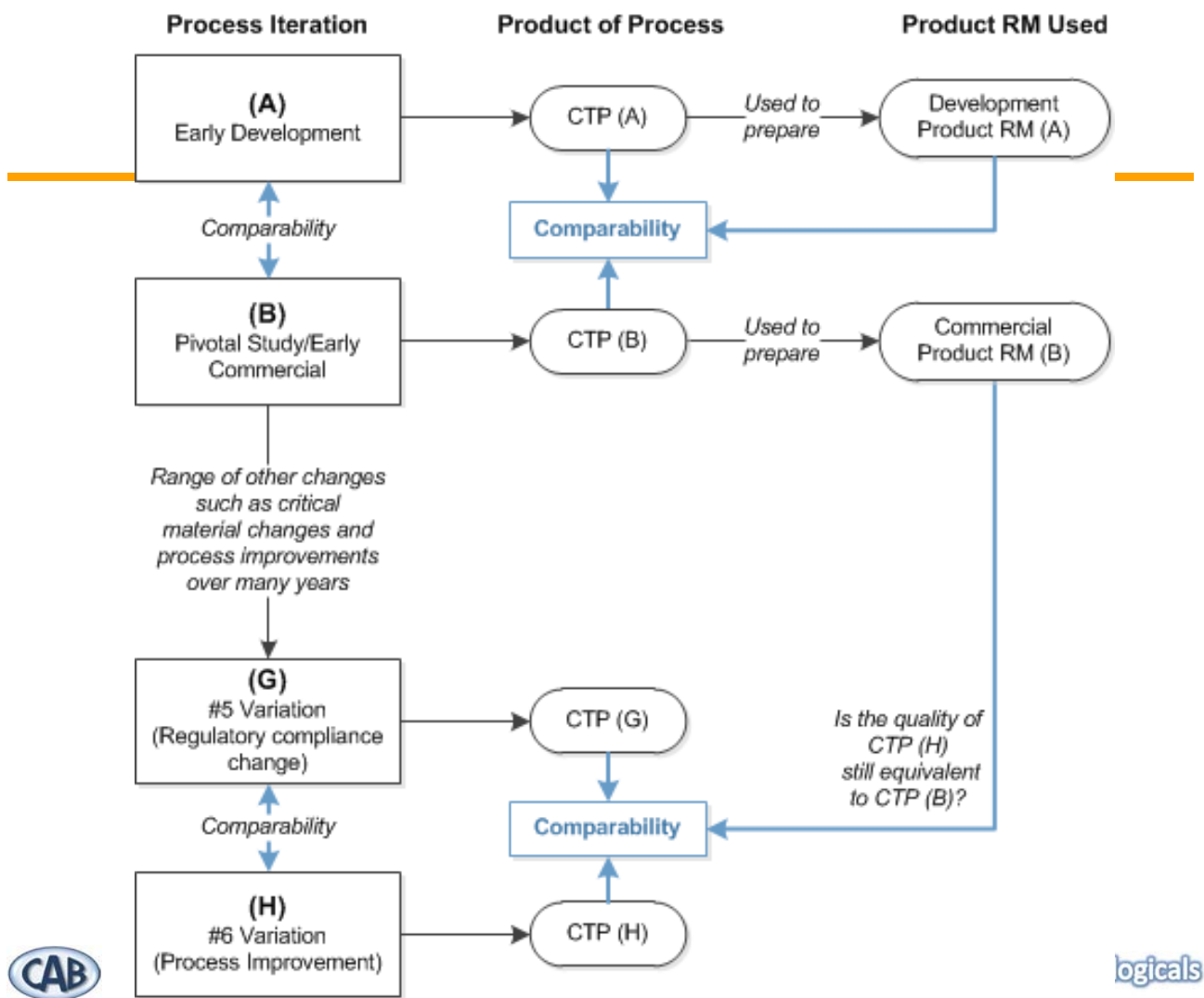
BUT, Does $A = D$?



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Conclusions

- Reference materials help to demonstrate measurement reliability over time
- Reference materials need to be **sufficiently similar to the material to be measured** to be reliable
- Thinking carefully about what is being measured (analyte and measurand) is important
 - The assay may define the measurand
- Preparation of a living cell RM (measurement or product) poses a range of issues
 - May require imaginative thinking