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

Christopher A Bravery

cbravery@advbiols.com

Overview

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

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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*

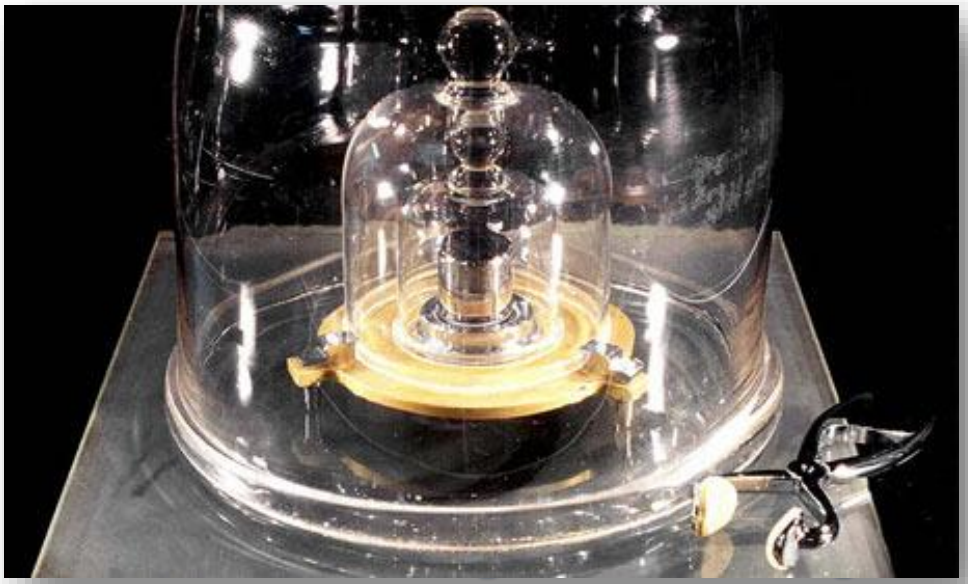
What is a US (imperial) pound?

- 0.45359237 Kg

So 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.



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/

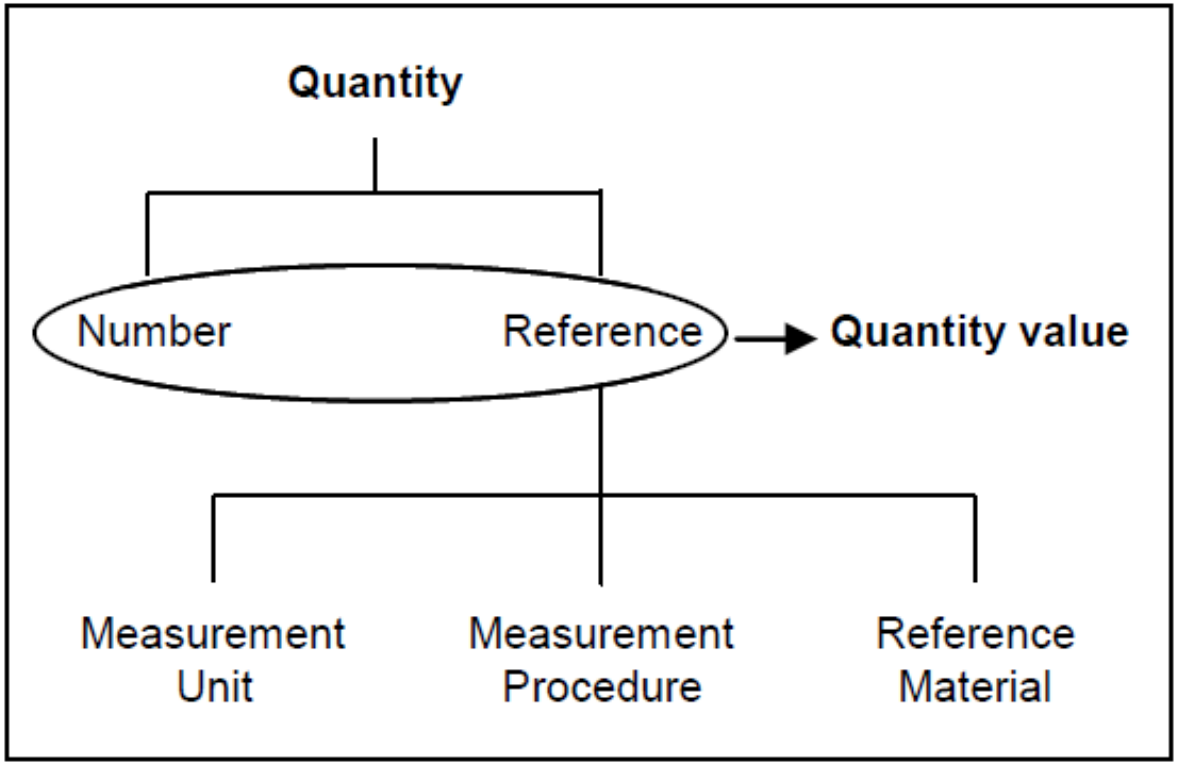
SI Base Units

- **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

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

Simple Example: protein in urine?

- **Analyte**: (total) protein.
- **Measurand**: Protein in urine.
- Is this useful?

- **Analyte**: (total) protein.
- **Measurand**: Protein in 24h urine.
- Is this more useful?

But measuring protein isn't simple

Table 2
Plasma protein concentration

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Sample (plasma), $n=7$	Protein concentration (graph) (mg/ml)	Protein concentration formula (BSA standard[a]) (mg/ml)	Protein concentration formula (HSA standard[b]) (mg/ml)
Amido Black			
Median±S.D.	83.4±1.93	82.6±1.91	94.9±2.16
CV %	2.3	2.3	2.3
Lowry			
Median±S.D.	110.2±4.09	109.1±4.31	80.3±2.57
CV %	3.7	3.9	3.2
Bradford			
Median±S.D.	95.1±3.3	89.5±3.1	72.5±2.5
CV %	3.5	3.5	3.5
Biüret			
Median±S.D.	88.8±3.54	80.9±3.67	81±3.98
CV %	4	4.5	4.9
Ponceau-S/TCA			
Median±S.D.	92.3±5.71	94.7±5.86	94.2±6
CV %	6.2	6.2	6.4

[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.

RM=HSA

RM=HSA
RM=BSA

RM=BSA

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

Potency Example: Identifying RM

- **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.

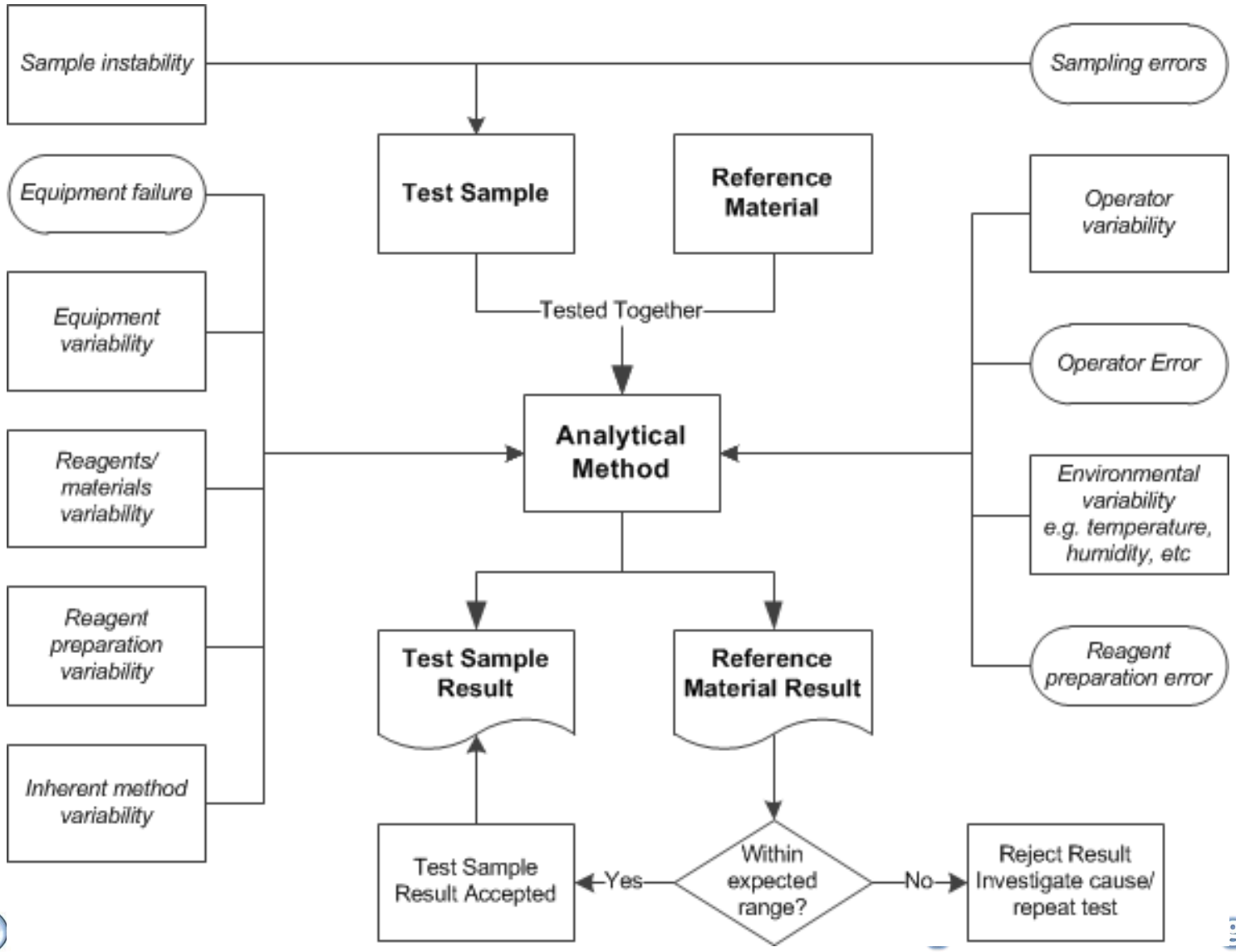
ENOUGH?

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.*

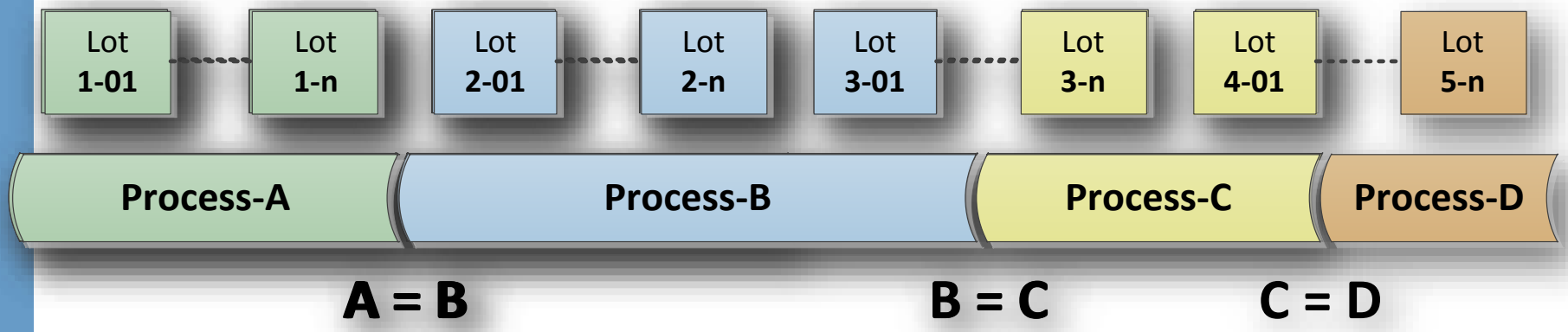
- Ideally here you would have a RM composed of cells that have been shown to secrete 'X' consistently in response to the stimulus in a way that is representative of the cell to be measured.

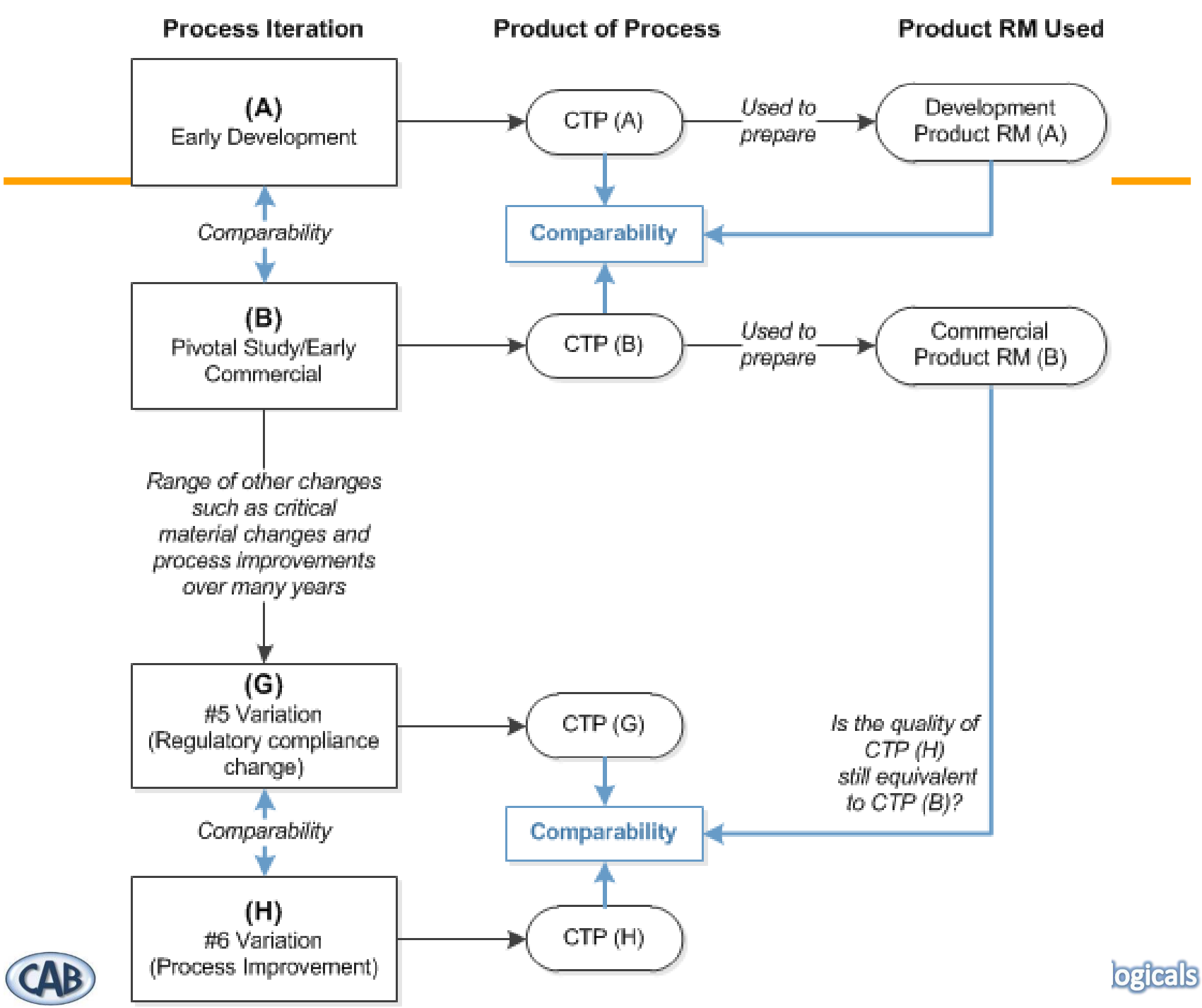
- But this is likely to be tricky; imaginative solutions may be needed,

Product Reference Materials

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

BUT, Does A = D ?




Process Iteration
Product of Process
Product RM Used
(A)
Early Development
CTP (A)
Used to prepare
Development Product RM (A)
Comparability
Comparability
(B)
Pivotal Study/Early Commercial
CTP (B)
Used to prepare
Commercial Product RM (B)
Range of other changes such as critical material changes and process improvements over many years
(G)
#5 Variation (Regulatory compliance change)
CTP (G)
Is the quality of CTP (H) still equivalent to CTP (B)?
Comparability
Comparability
(H)
#6 Variation (Process Improvement)
CTP (H)

Conclusions

- Reference materials help to demonstrate measurement reliability over time
- Reference materials need to be **sufficiently similar to the material to me 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 loving cell RM (measurement or product) poses a range of issues
  - May require imaginative thinking