Certified Reference Materials and Measurement Uncertainty

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**Joint Research Centre
IRMM**

Serving society
Stimulating innovation
Supporting legislation

European Commission

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JRC IRMM

Scene setting
CRM development
Intended use
Traceability
Measurement uncertainty
Alternatives

European
Commission

IRMM's Reference Materials Programme

Vitamins

Mycotoxins

PCBs

Proximates

Veterinary drugs

Prions

Food CRMs

Enzymes

TSE QCMs

Clinical CRMs

Hormones

Microorganisms

Proteins

PAHs

Microbiol. CRMs

GMO CRMs

Hydrocarbons

DNA

Dioxins

Soil/ Sediment CRMs

Engin. Mat. CRMs

Size

Elements

Ions

Nuclear CRMs

Water CRMs

Impact toughness

Isotopes

Metal species

BELAC

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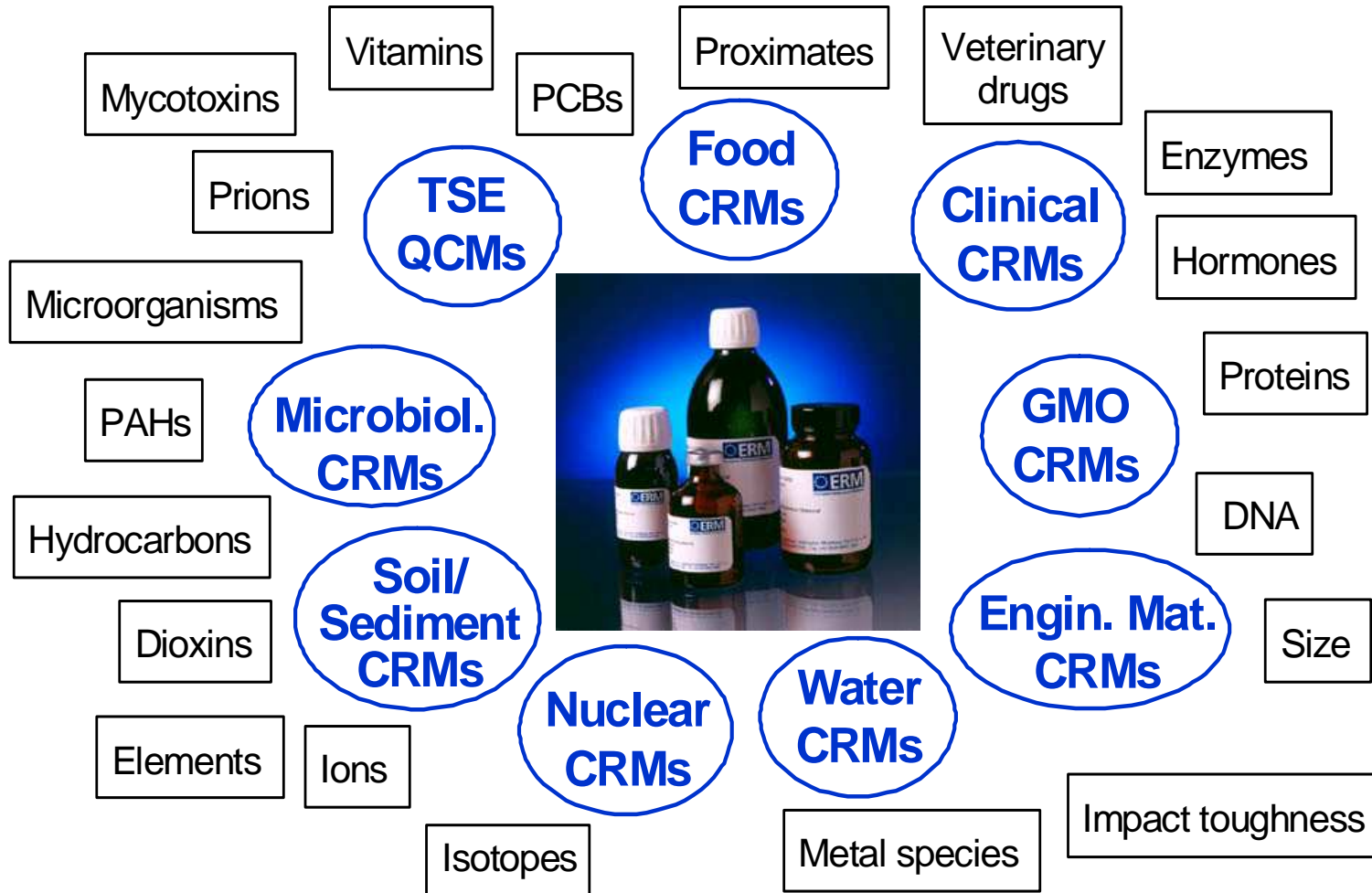
ISO Guide 34 accreditation (BELAC No. 268-RM)
ISO/IEC 17025 accreditation (BELAC No. 268-TEST)

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GMO CRMs activities

Development of certified reference materials (CRMs) on request of the **Biotech Industry**

Intellectual property right (IPR) issues are agreed within a material transfer agreement (MTA) signed by IRMM and the IPR owner (http://irmm.jrc.ec.europa.eu/reference_materials_catalogue/related_services/Pages/index.aspx)

Research and development
for a typical GMO CRM
takes about 14 months

Specific situation in Europe

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European Commission

GMO events need authorisation

before being placed on the market in Europe
(Regulation (EC) No 1829/2003)

Quantification method and certified reference material (CRM)

need to be made available to GMO testing laboratories world-wide
(Regulation (EC) No 1829/2003, (EU) No 619/2011)

Certified reference material (CRM)

are developed on request of the Biotech Industry in the frame of
the authorisation request

Measurements results can be expressed in different units

while Recommendation 2004/787/EC suggests copy number
ratios (as measured by qPCR), Regulation (EU) No 619/2011)
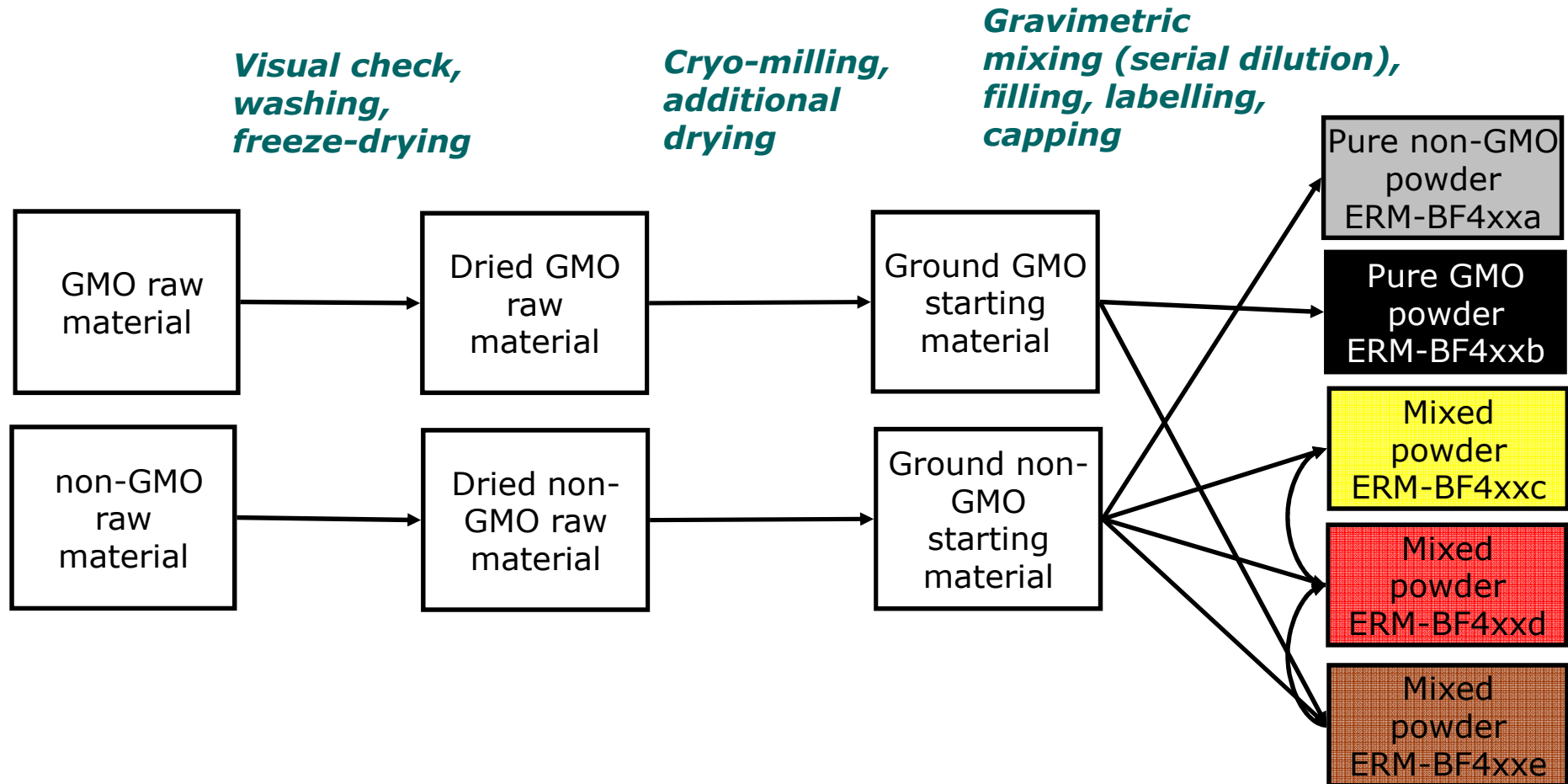demands the expression in mass fractions

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CRM Development
(Processing + Certification = Reference Material
with certified value and uncertainty)

Processing

Scene setting
CRM development
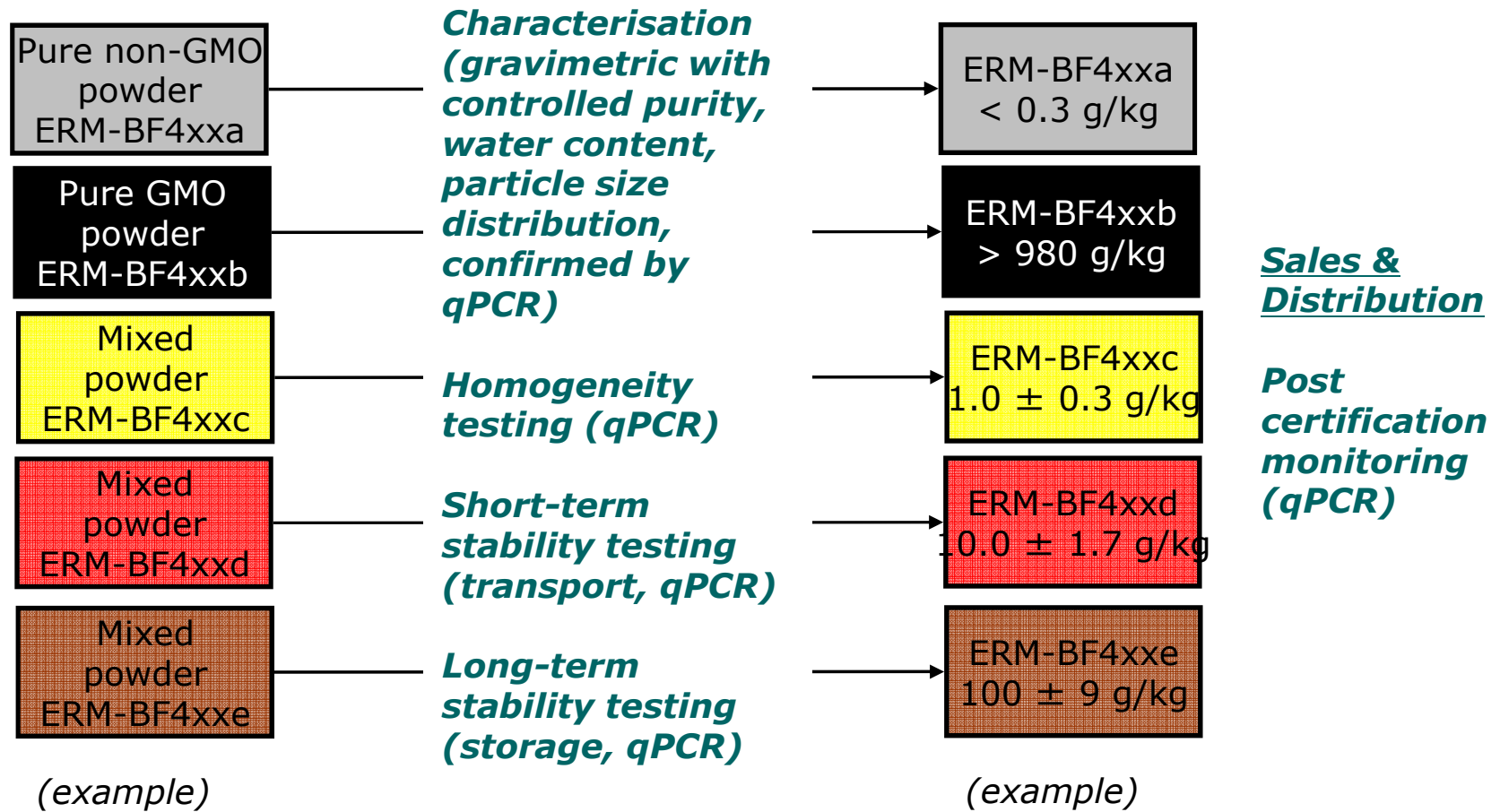Intended use
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Visual check, washing, freeze-drying

Cryo-milling, additional drying

Gravimetric mixing (serial dilution), filling, labelling, capping

GMO raw material → Dried GMO raw material → Ground GMO starting material

non-GMO raw material → Dried non-GMO raw material → Ground non-GMO starting material

Pure non-GMO powder ERM-BF4xxa

Pure GMO powder ERM-BF4xxb

Mixed powder ERM-BF4xxc

Mixed powder ERM-BF4xxd

Mixed powder ERM-BF4xxe

(example)

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Certification

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Pure non-GMO powder ERM-BF4xxa	*Characterisation (gravimetric with controlled purity, water content, particle size distribution, confirmed by qPCR)*	ERM-BF4xxa < 0.3 g/kg
Pure GMO powder ERM-BF4xxb		ERM-BF4xxb > 980 g/kg
Mixed powder ERM-BF4xxc	*Homogeneity testing (qPCR)*	ERM-BF4xxc 1.0 ± 0.3 g/kg
Mixed powder ERM-BF4xxd	*Short-term stability testing (transport, qPCR)*	ERM-BF4xxd 10.0 ± 1.7 g/kg
Mixed powder ERM-BF4xxe	*Long-term stability testing (storage, qPCR)*	ERM-BF4xxe 100 ± 9 g/kg

Sales & Distribution

Post certification monitoring (qPCR)

(example)   *(example)*

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Intended use of GMO CRMs
(calibration and quality control)

Calibration and quality control

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Quality control

(or)

Calibration

Food/feed sample → Sample preparation → DNA extraction / purification → Real-time PCR → Data evaluation → Measurement result

cryIA(b)

$y = -3.217x + 37.667$
$R^2 = 0.9954$

hmg

$y = -3.301x + 39.259$
$R^2 = 0.999$

cryIA(b)

$y = -3.217x + 37.667$
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hmg

$y = -3.301x + 39.259$
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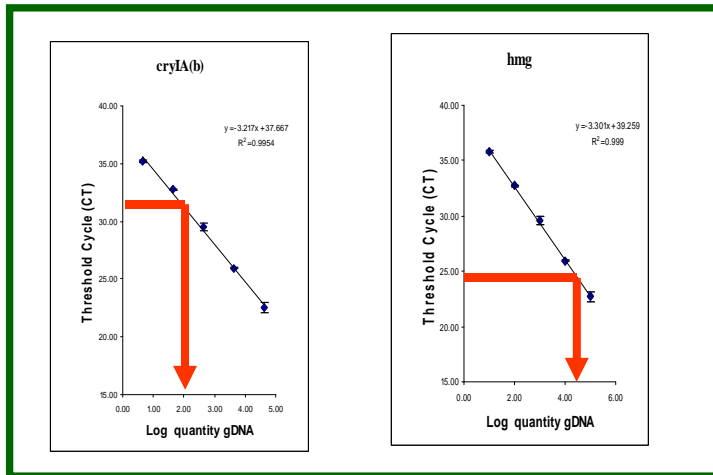
Quality control use
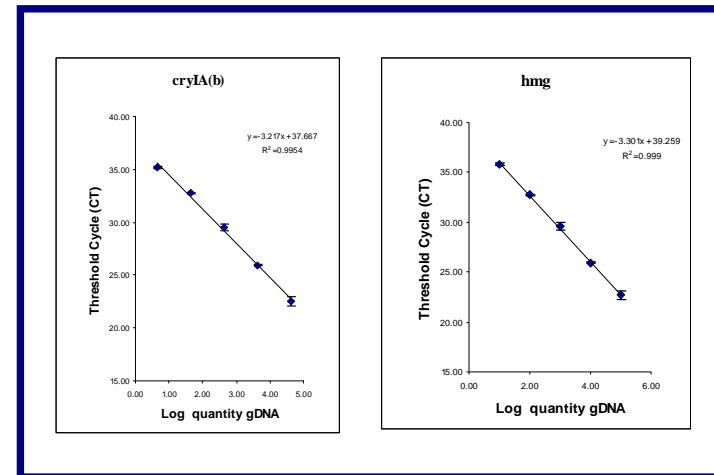
Setting up of calibration curves

ERM® Application Note 5 (http://www.erm-crm.org)

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Quality control
- control charts

Shewhart control chart (most popular)

- **Central line**
  = mean value (min n = 10)
- **Upper/lower warning limits**
  = mean value $\pm$ 2 · s_{QC}
- **Upper/lower control limits**
  = mean value $\pm$ 3 · s_{QC}
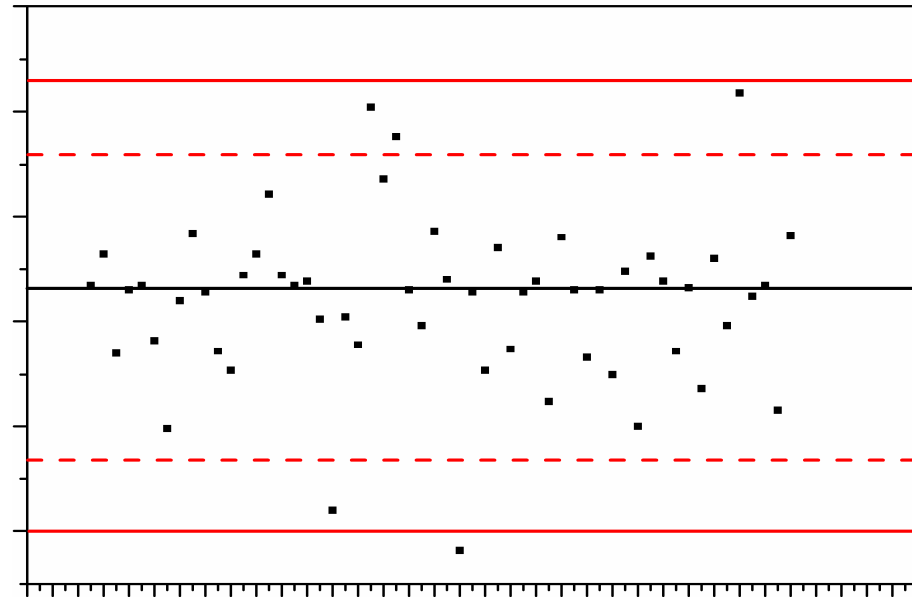
Out-of-control

- 1 point above/below control limits
- 2 out of 3 consecutive points between warning and control limits
- 9 consecutive points on the same side of the central line
- 6 or more points in a row steadily increasing or decreasing
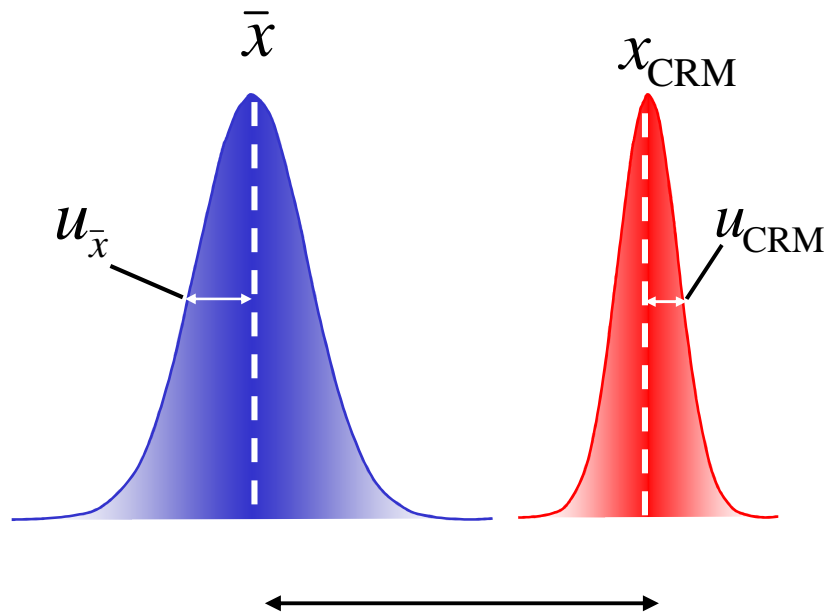 *(based on ISO 8258:1991; other interpretations exist)*

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Trueness
(bias control)

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Quality control – Bias control (I)
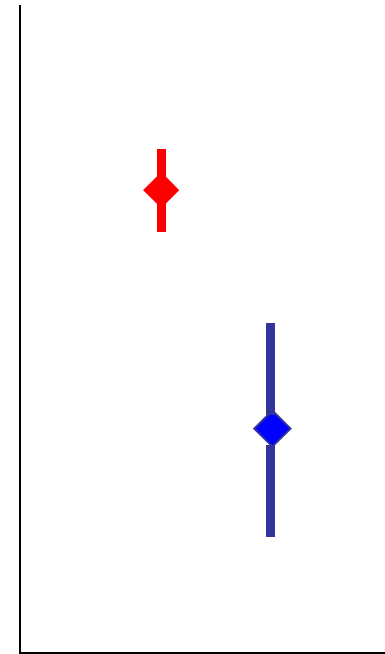

test results   certified value

$\bar{x}$

x_{CRM}

$u_{\bar{x}}$
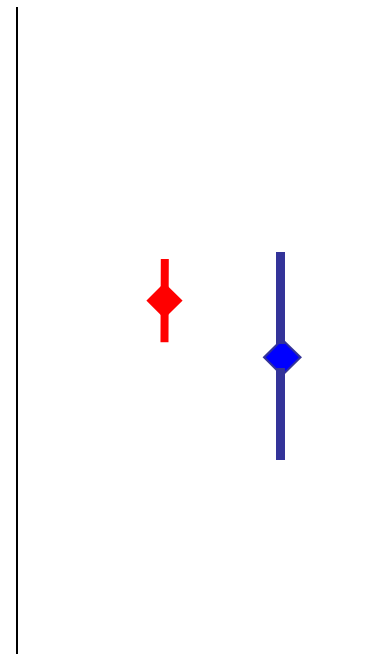
u_{CRM}

bias

unbiased   biased

ERM® Application Note 1 (http://www.erm-crm.org)

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Quality control – Bias control (II)

Multi-step approach:

1. Determine difference (Δ_{m}) between mean measured value (c_{m}) and certified (true) value (c_{CRM})

$$\Delta_{\mathrm{m}} = \left| c_{\mathrm{m}} - c_{\mathrm{CRM}} \right|$$

2. Convert expanded uncertainty (U_{CRM}) of c_{CRM} into standard uncertainty (u_{CRM})

$$u_{\mathrm{CRM}} = \frac{U_{\mathrm{CRM}}}{k}$$

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Quality control – Bias control (II)

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3. Estimate measurement uncertainty (u_{m})

   One option: within-laboratory standard deviation (intermediate precision via control chart based on CRMs)

4. Estimate the combined uncertainty (u_{Δ})

$$u_{\Delta} = \sqrt{u_{\mathrm{m}}^2 + u_{\mathrm{CRM}}^2}$$

5. Compare Δ with $2 \cdot u_{\Delta}$

$$\text{if} \, \Delta \leq 2u_{\Delta}$$ **the method not significantly biased**!

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Measurement uncertainty

What's measurement uncertainty?

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ISO 'GUM' definition (*ISO Guide 98-3*):

A parameter, associated with the <u>result</u> of a measurement, that characterises the <u>dispersion of the values</u> that could reasonably be attributed to the <u>measurand</u>

<u>Interval, that covers the true value </u>with a high probability

The part of the result after the ±

Distinguish between uncertainty and error (bias)!

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Various ways to estimate MU

Most practical (top-down approaches):

Using CRMs
(*ERM® Application Note 4,* http://www.erm-crm.org)
or in-house samples
(Guidance document on Measurement Uncertainty for GMO Testing Laboratories,
http://irmm.jrc.ec.europa.eu/reference_materials_catalogue/user_support/Documents/eur22756en.pdf)

Make use of validation/verification data, data from quality control charts and routine analysis!

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Looking for alternatives

What to use?

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- Matrix CRMs with certified values close to the decision threshold(s)

- Ground matrix CRMs certified for purity *(requiring weighing and uncertainty estimations for the mixtures produced)*

Example

- Seed CRMs certified for purity *(requiring grinding, weighing and uncertainty estimations for the mixtures produced)*

- In-house samples without certified values *(requiring information about the stability, homogeneity and true value)*

Increasing work load!

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Ground matrix CRMs certified for purity (I)

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Example:

Pure GM material > 985 g/kg (95 % probability)
Pure non-GM material < 1 g/kg (95 % probability)

Weighing: 0.01 g GM powder 0.99 g non-GM powder

MU: arising from the purity of the non-GM material and
GM material
arising from the weighing of the non-GM material
and GM material

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Ground matrix CRMs certified for purity (II)

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$$u = \sqrt{u_{p(1)}^2 + u_{p(2)}^2 + u_{w(1)}^2 + u_{w(2)}^2}$$

u combined standard uncertainty

$u_{p(1)}$ standard uncertainty related to the purity of the non-GM material

$u_{p(2)}$ standard uncertainty related to the purity of the GM material

$u_{w(1)}$ standard uncertainty related to the weighing of the non-GM material

$u_{w(2)}$ standard uncertainty related to the weighing of the GM material

$$u = \sqrt{(0.99\,\mathrm{g} \times 0.015)^2 + (0.01\,\mathrm{g} \times 0.001)^2 + (0.99\,\mathrm{g} \times 0.013)^2 + (0.01\,\mathrm{g} \times 0.013)^2}$$

The true value and combined (rounded) standard uncertainty is
1.00 ± 0.13 g/kg

Note: Suitable for bias control, expanded uncertainties need to be multiplied with the appropriate coverage factor k!

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Avoiding pitfalls

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Ground matrix CRMs certified for purity

- equilibrate the non-GM an GM material to the same water content (place for 24 h in an open dish)
- assure that balance used has an appropriate uncertainty (analytical balance)
- Extract from the whole material to avoid inhomogeneous material

Seed CRMs certified for purity

- *additionally check that similar particle sizes are obtained after grinding*
- *mix at the level of DNA if the above cannot be guaranteed and check for potential matrix effects by spiking into extracts from non-GM material*

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Further (selected) information

Guidance document on Measurement Uncertainty for GMO Testing Laboratories:
http://irmm.jrc.ec.europa.eu/reference_materials_catalogue/user_support/Documents/eur22756en.pdf

ERM® Application Notes: http://www.erm-crm.org

Guide to the Expression of Uncertainty in Measurement (GUM, ISO/IEC Guide 99:2007):
http://www.bipm.org/en/publications/guides/gum.html

Handbook for the calculation of measurement uncertainty in environmental laboratories
http://www.inn.cl/pdfs/incertidumbre/Nordtest%20Handbook%20Uncertainty.pdf

and many more…… (i.e. ISO/REMCO working on a Guide for in-house reference materials)

Training

Use of reference materials and the estimation of measurement uncertainty Training course

09-10 October, 2013 IRMM, Geel

This course provides participants with the theoretical basis for the estimation of measurement uncertainty and establishment of traceability. The course is intended for laboratory managers and practitioners in analytical laboratories who use reference materials for statistical quality control, method validation and calibration and need to assess measurement uncertainties on customer's demand or as requirement of ISO/IEC 17025.

http://irmm.jrc.ec.europa.eu/training/Pages/index.aspx

Information leaflets available!

IRMM GMO CRMs

ERM-BF410k (RUR soya)
ERM-BF411(Bt-176 maize)
ERM-BF412 (Bt-11 maize)
ERM-BF413k (MON 810 maize)
ERM-BF414 (GA21 maize)
ERM-BF415 (NK603 maize)
ERM-BF416 (MON 863 maize)
ERM-BF417 (MON 863 x MON 810 maize)
ERM-BF418 (1507 maize)
ERM-BF419 (H7-1 sugar beet)
ERM-BF420 (3272 maize)
ERM-BF421 (EH92-527-1 potato)
ERM-BF422 (281-24-236 x 3006-210-23 cotton)
ERM-BF423 (MIR604 maize)
ERM-BF424 (59122 maize)
ERM-BF425 (356043 soya)
ERM-BF426 (305423 soya)
ERM-BF427 (98140 maize)
ERM-BF428 (GHB119 cotton)
ERM-BF429 (T304-40 cotton)
ERM-BF430 (AM04-1020 potato)
ERM-BF431 (AV43-6-G7 potato)
ERM-BF432 (DAS-684164 soya)
ERM-BF433 (DAS-40278-9 maize)
ERM-BF434 (73496 rapeseed)
ERM-BF435 (PH05-026-0048 potato)
ERM-BF436 (DAS-44406-6 soya)

IRMM GMO CRMs:
http://irmm.jrc.ec.europa.eu/
(listed here)

AOCS GMO CRMs:
https://secure.aocs.org/crm/
(not listed here)

More detailed information about the GMO CRMs is available via Internet (certificates and reports)!

upcoming

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Thank you for your attention