EU-China-Safe training event

Reference Materials

Prof Michael Walker

Agenda

- What are reference materials and why do we need them?
  - The differences between reference materials (RM) and certified reference materials (CRM)
- How should reference materials be made?
  - ISO 17034 :2016 General requirements for the competence of reference material producers
- What information should accompany RM and CRM
- Selection of RM and CRM
- Handling and storage
- Use in method validation and analytical quality control
- Sources of further information, training and guidance

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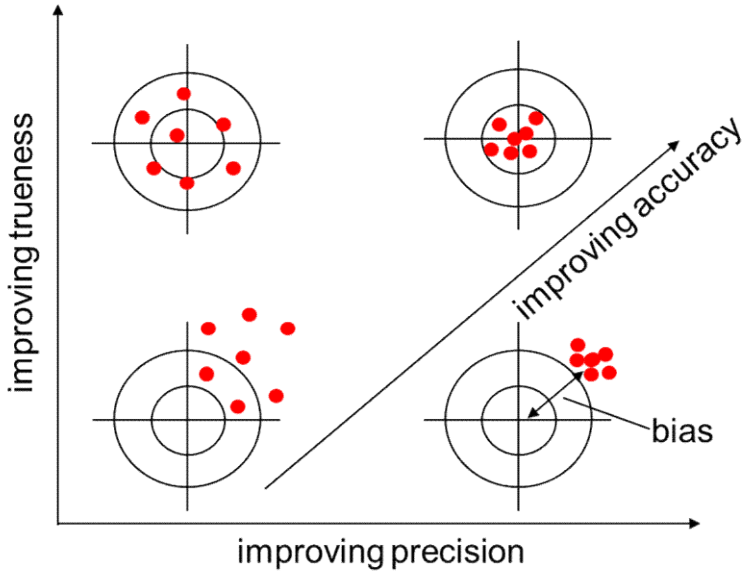
The importance of analytical results

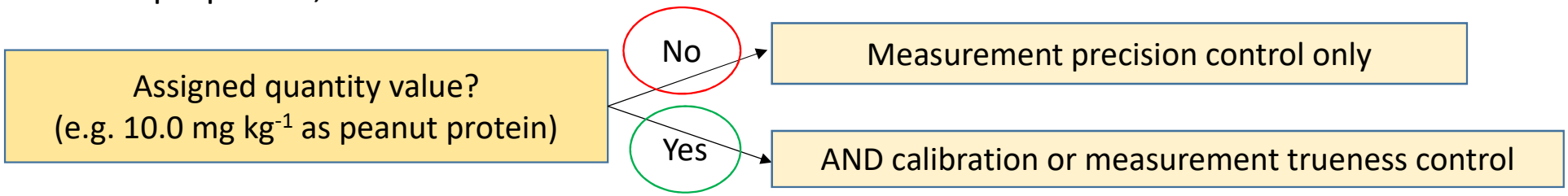
Reference materials - why do we need them?

- All measurements involve comparing an ‘unknown’ (e.g. the test sample) with a reference, (e.g. the calibrator)
- A reference material is applied:
- To support **measurement quality** through
  - Calibration, method validation, estimation of measurement uncertainty, training, internal QC and external QA (PT)
- In a **wide variety of fields**:
  - Chemical and biological composition, safety and authenticity, clinical, physical, engineering properties and miscellaneous areas such as taste and odour.
- May be **characterised** for:
  - ‘identity’ (e.g. chemical structure, fibre type, microbiological species etc.) or
  - ‘property values’ (e.g. amount of specified chemical entity, hardness etc.).



Reference materials, RM – what are they?

- VIM 5.13 (6.13) **reference material RM**:
  - material, sufficiently **homogeneous** and **stable** with reference to one or more specified properties, which has been established to be **fit for its intended use in measurement**



  - In a given measurement, a given reference material ideally should only be used for either calibration or quality assurance.
- Examples:
  - Pooled human serum (no assigned value for e.g. cholesterol concentration →precision control only;
  - Fish tissue containing a stated mass fraction of a dioxin, may be used as a calibrator.
  - *Example of reference material embodying nominal properties*: colour chart …

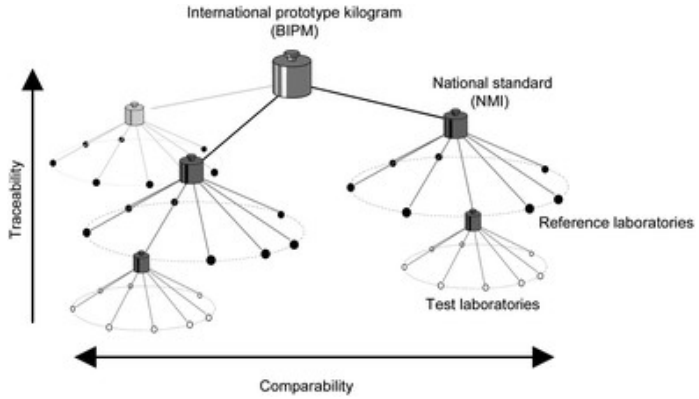
International vocabulary of metrology — Basic and general concepts and associated terms (VIM) /
Vocabulaire international de métrologie — Concepts fondamentaux et généraux et termes associés (VIM)
https://www.iso.org/sites/JCGM/VIM/JCGM_200e.html

Some commonly encountered types of RM

- **Pure substances** characterised for chemical purity and/or trace impurities.
- **Standard solutions and gas mixtures**, often prepared gravimetrically from pure substances and used for calibration purposes.
- **Matrix reference materials**, characterised for the composition of specified major, minor or trace chemical constituents. Such materials may be prepared from matrices containing the components of interest, or by preparing synthetic mixtures.
- **Physicochemical reference materials** characterised for properties such as melting point, viscosity, and optical density.
- **Reference objects or artefacts** characterised for functional properties such as taste, odour, octane number, flash point, hardness, microscopy specimens e.g. characterised for fibre type, and microbiological specimens.

Certified reference materials

- VIM 5.14 (6.14) **certified reference material CRM**:
  - reference material, accompanied by documentation issued by an authoritative body and providing one or more specified **property values with associated uncertainties and traceabilities**, using valid procedures



(PS - Just because it has a certificate doesn't mean it's a CRM)

New set of GM cotton certified reference materials released

15 July 2021

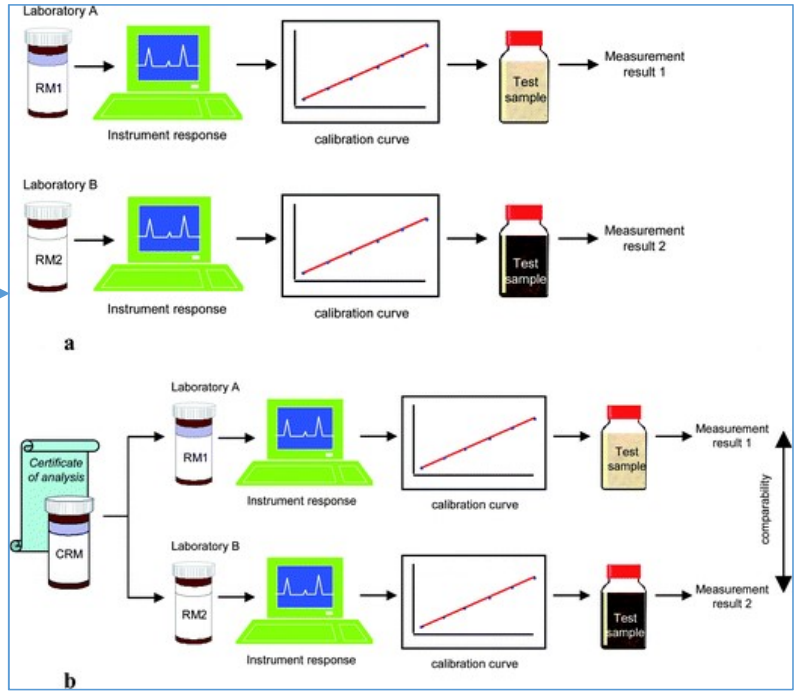
The JRC developed a new set of GMO certified reference materials (CRMs). The five CRMs are certified for their mass fraction of GHB811 cotton. After authorisation of the GMO event in food and feed products for the EU market, the CRMs will enable the use of the GMO quantification method for GHB811 cotton and the implementation of EU legislation on GMO labelling.

The GHB811 cotton (with unique identifier code BCS-GH811-4), developed by BASF Agricultural Solutions Seed LLC (US), has been modified for resistance to the herbicides glyphosate and isoxaflutole. The double mutant 5-enol pyruvylshikimate-3-phosphate synthase (2mepsps) gene that encodes for the 2mEPSPS protein

Barwick & Wood, 2010 J Analyt Atomic Spect, 25, 785-799.

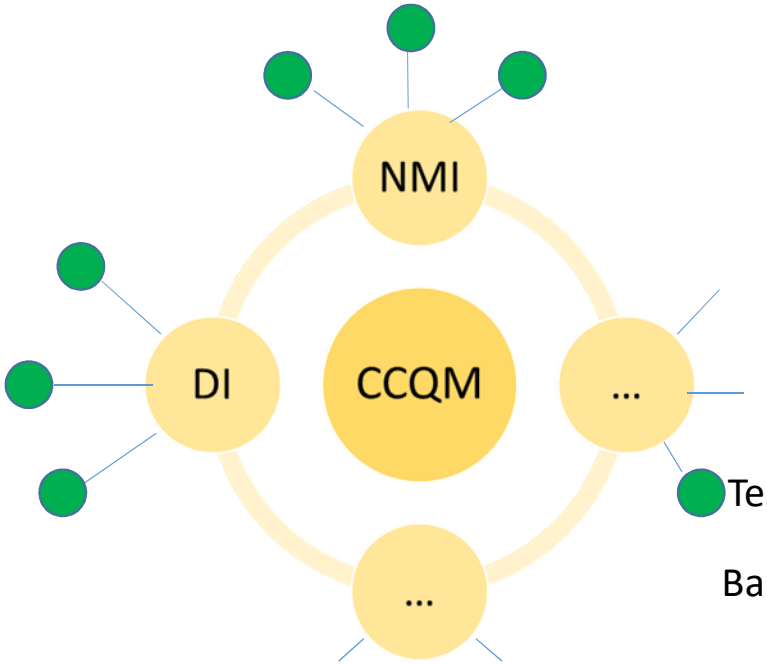
Metrological traceability…

- …is the property of a measurement result which allows measurements made under different conditions (e.g. at different times, by different people, in different locations, using different measurement procedures) to be compared in a meaningful way.
- The infrastructure that supports measurements of mass, length and time is well established …
- What about chemical & biochemical measurements?
- For laboratories to establish the metrological traceability of their results it is essential that the materials they use during calibration and method validation are traceable to a higher level.



(Bio)chemical global measurement infrastructure …

- … established by the BIPM, through the Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM)



Test laboratories

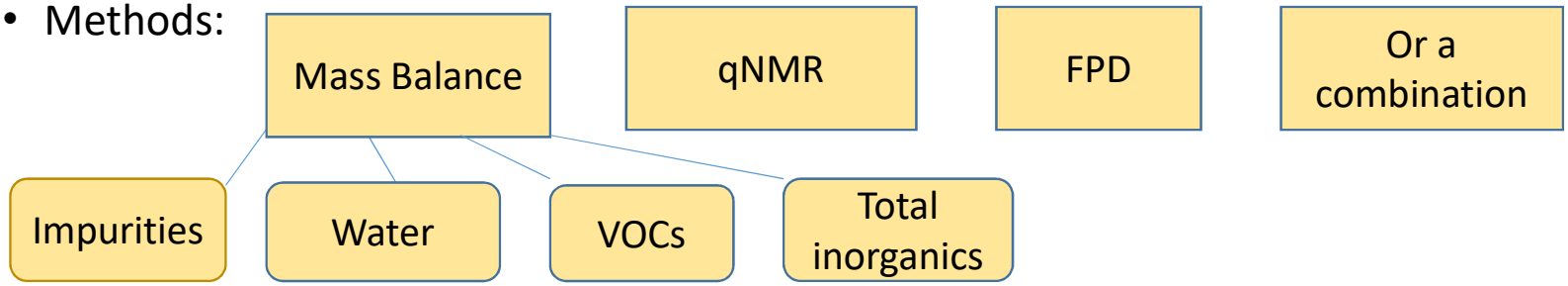
https://www.bipm.org/en/committees/cc/ccqm

CCQM coordinates the work of the National Measurement Laboratories, NMIs and other designated institutes DIs

Barwick & Wood, 2010 J Analyt Atomic Spect, 25, 785-799.

‘Key Comparison’ studies

- Interlaboratory studies - assess and demonstrate the measurement capabilities of the NMIs & DIs
- Establish the equivalence of their measurement standards.
- Provide metrological traceability to testing laboratories via their analytical and calibration facilities, & the production and sale of pure substance and matrix certified reference materials.
- Example: Mass fraction assignment of Bisphenol-A high purity material, CCQM-K148
- Participants were required to report the mass fraction of Bisphenol A in one supplied unit of the comparison material, homogeneity & stability assessed prior to issue.
- Methods:



https://www.bipm.org/documents/20126/48766255/OAWG_20_064.pdf/57078e08-66ed-2630-04a1-03ec2a8bd7e3

Bisphenol-A high purity material, CCQM-K148

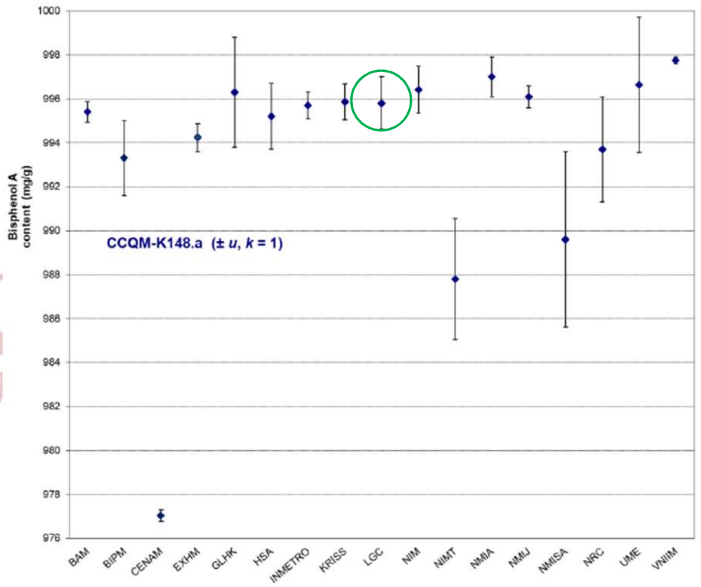


Figure 2: Participant Results for BPA content in CCQM-K148.a
Reported results for BPA content in the CCQM-K148.a material by participant sorted by alphabetical ord the NMI. Dots represent the reported value, x; bars the standard uncertainty, $u(x)$, in units of mg/g.

NMI	x (mg/g)	$u(x)$ (mg/g)	$u_{rel}(x)$ (%)	k	$U_{95}(x)$ (mg/g)	$U_{rel}(x)$ (%)
BAM	995.4	0.46	0.046	2	0.91	0.091
BIPM	993.3	1.7	0.171	2	3.4	0.34
CENAM	977.02	0.26	0.027	2	0.52	0.052
EXHM	994.23	0.64	0.64	2	1.28	0.13
GLHK	996.3	2.5	0.251	2	4.9	0.49
HSA	995.2	1.5	0.151	2	3.0	0.30
INMETRO	995.7	0.6	0.060	2	1.2	0.12
KRISS	995.87	0.82	0.082	2.45	2.02	0.202
LGC	995.8	1.2	0.121	2	2.5	0.25
NIM	996.41	1.08	0.108	2	2.17	0.217
NIMT	987.8	2.76	0.279	2	5.60	0.56
NMIA	997	0.9	0.090	2.3	2.0	0.20
NMIJ	996.1	0.50	0.050	2	1.00	0.10
NMISA	989.6	4.0	0.404	2	8.0	0.80
NRC	993.7	2.4	0.242	2	4.8	0.48
UME	996.64	3.03	0.304	2	6.06	0.606
VNIIM*	997.75	0.146	0.015	2	0.29	0.029

Table 4: Reported results for BPA content in CCQM-K148.a (mg/g)

The Key Comparison Reference Value for the BPA content was assigned using a Hierarchical Bayesian Random Effects Model (HB REM) estimator from the values reported and was 995.7 ± 0.6 mg/g (99.57 ± 0.06% wt/wt)

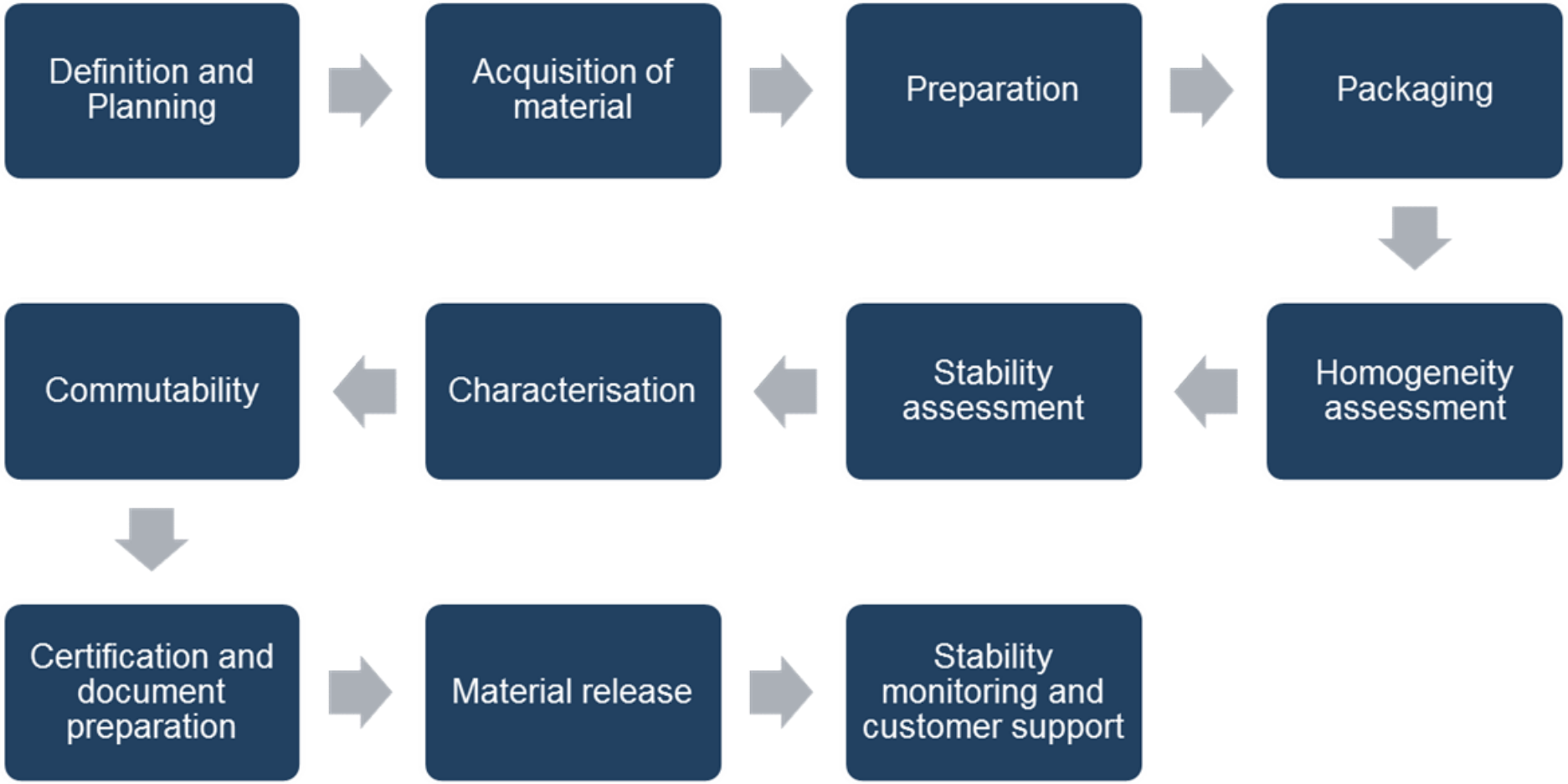
How should reference materials be made?

- CCQM studies establish higher order reference standards
- RMs & CRMs produced in that way require exceptionally high levels of expertise, budget and international collaboration by the NMIs and DIs.
- ‘Routinely’ produced RMs would be prohibitively expensive if produced in the same way,
- However, a high degree of rigour must be involved
- The most reliable RMs and CRMs are produced by
  - NMIs and DIs, and/or
  - Organisations accredited to ISO 17034 :2016 General requirements for the competence of reference material producers and/or
  - have a peer reviewed publication on the preparation and characterisation of the RM or CRM
- RM production is thus seldom a commercial proposition

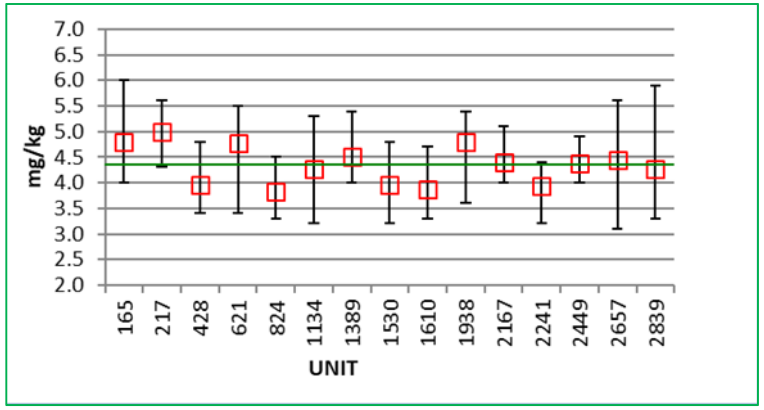
ISO 17034 :2016 General requirements for the competence of reference material producers

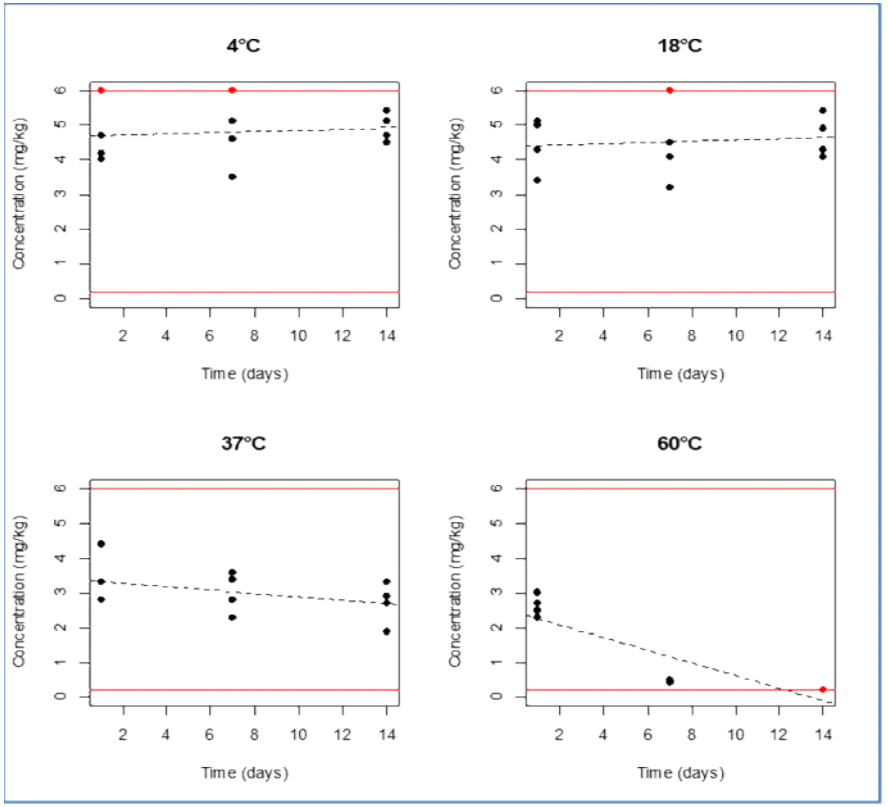
- This International Standard outlines the general requirements for the producers of RMs, including certified reference materials (CRMs).
- It supersedes ISO Guide 34:2009 and is aligned with the relevant requirements of ISO/IEC 17025.
- Further guidance
  - ISO Guide 31 and ISO Guide 35. (e.g. concerning the content of certificates and the design of characterization, homogeneity and stability studies)
- ISO 17034 :2016 covers:
  - General requirements - Contractual matters, Impartiality, Confidentiality
  - Structural and resource requirements (e.g. personnel, subcontracting, equipment, services and supplies, facilities and environmental matters,
  - Technical and production requirements, (e.g. production planning and control, material handling, processing and storage, measurement equipment & procedures
  - Data integrity and evaluation, metrological traceability of certified values, assessment of homogeneity and stability, characterisation, assignment of values and uncertainty, RM documents and labels, distribution, control of records, management of non-conforming work, complaints, quality policy, management review, internal audit, risks and opportunities, corrective actions/improvement, customer feedback.

Steps in the production of a reference material



Homogeneity and stability - example





What information should accompany an RM

- Ideally, a certificate of analysis complying with ISO 17034 :2016 and a report covering the characterisation, certification and statistical analysis procedures, should be available.
- However, many RMs, particularly materials not specifically produced as RMs, may not fully comply with ISO 17034 :2016
- Alternative, equivalent information in whatever form it is available, that provides credible evidence of fitness for purpose may be considered acceptable.
  - For example: technical reports, trade specifications, a peer reviewed publication or reports of scientific meetings or correspondence with suppliers.
- EXAMPLE
  - LGC Standards: Aflatoxin B_1 https://www.lgcstandards.com/GB/en/Aflatoxin-B1/p/DRE-C10047100
    - Synonyms, Product Code, CAS no. Molecular formula and molecular weight, pack size
  - Certificate of analysis
  - Safety data sheet

REFERENCE MATERIAL CERTIFICATE

Aflatoxin B1

1. General information

This document is designed and the certified value(s) and uncertainty(ies) are determined in accordance with ISO Guide 31 [1] and Eurachem / CITAC Guides [2,3].

2. Description of the Reference Material (RM)

Name:	Aflatoxin B1
CAS number:	1162-65-8
Catalog number:	DRE-C10047100
Lot #:	S17273B
Certificate version:	1
Expiry date:	05.07.2022
Physical description of RM:	White crystals of Aflatoxin B1
Packaging and amount of RM:	Amber glass ampoules fitted with teflon faced butyl septa and PP screw caps, quantity of 5 mg of RM
Name and address of the manufacturer:	Romer Labs Diagnostic GmbH Technopark 5, 3430 Tulln, Austria www.romerlabs.com
Name and address of the supplier:	LGC Standards GmbH Mercatorstraße 51, 46485 Wesel, Germany Tel +49(0)2 81 98 87 0, Fax +49(0)2 81/98 87 199 www.lgcstandards.com

2.1 Intended use of the RM

- for laboratory use only
- calibration of analytical instruments

2.2 Instruction for the correct use of the RM

The ampoules should be stored at 2-8°C in a dark place. Before usage of the RM, the ampoules should be allowed to warm to room temperature. The recommended minimum sub-sample amount for all kinds of application is 1 mg. The expiry date of this RM is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/packages. Solutions prepared for calibration purposes should be protected from exposure to light. Discard solutions after use in accordance with appropriate safety regulations for chemical substances.

2.3 Hazardous situation

The normal laboratory safety precautions should be observed when working with this RM. Further details for the handling of this RM are available as safety data sheet (SDS).

3. Certified values and their uncertainties

Aflatoxin B1		
Compound	**Purity**	
	Certified value [a]	Uncertainty [b]
Aflatoxin B1	98.8 %	± 1.2 %

[a] *The certified value is based upon the results from several analytical techniques*

[b] *Expanded uncertainty U (k = 2) of the value u_c according to GUM [4]*

3.1 Calculation of the certified value and discussion of uncertainty

The purity check with LC-DAD and UV-spectrophotometry showed a purity with an estimated mass concentration of total 98.0 % of the investigated sample. Based on these findings, maximum impurity level in solid Aflatoxin B1 can be estimated with 2.0 %.

To cover this range, an approach with an estimated purity of 98.8 % with a symmetrical uncertainty of ± 1.2 % was used according to a procedure which has been accepted by the European Commission within a Standards, Measurements and Testing (SMT) project [5]. The conservative assumption of a rectangular distribution was made and the tolerance of 1.0 % divided by $\sqrt{3}$ resulting in an uncertainty (u_c) of the presented purity level of 0.6 %. Following the Guide to the Expression of Uncertainty in Measurement (GUM) [1] the expanded uncertainty of the Aflatoxin B1 purity level is obtained by multiplication with a coverage factor k for which 2 is usually chosen to obtain a confidence level of approx. 95 %. Using this procedure a theoretical value for the purity of the crystalline Aflatoxin B1 sample of 98.8 % and its respective expanded uncertainty of ± 1.2 % can be calculated.

4. Discussion of traceability

The certified value (purity of Aflatoxin B1) is based on the result of LC-DAD analysis which was previously used as method for purity assessment of solid mycotoxins [5]. High purity material represents a practical realization of concentration units, through conversion of mass to molar quantity.

5. Purity assessment of Aflatoxin B1

5.1. UV-spectrophotometry

The wavelength scale accuracy in both UV and visible regions of the applied spectrophotometer was controlled with holmium oxide in dilute perchloric acid [6]. The absorbance scale and the linearity of the apparatus were validated with potassium dichromate in dilute sulfuric acid [7]. All measurements were performed at 22 ± 3 °C.

The UV absorption spectrum of Aflatoxin B1 was consistent with literature data [8] and showed no detectable impurities.

molecular mass	m	=	312.27	g/mol
molar absorptivity in ACN [a]	ε	=	20700	L mol^{-1} cm^{-1}
wavelength	λ	=	355	nm
dilution factor	f	=	2	
absorbance at λ = 335 nm [b]	A	=	0.776	
optical path length	d	=	1	cm
mass concentration [c]	c_m		23.5	± 0.3 µg/mL
analytical concentration [d]	c	=	23.4	± 0.4 µg/mL

$$c = \frac{A \times m \times 1000 \times f}{\varepsilon \times d} = \frac{0.776 \times 312.27 \times 1000 \times 2}{20700 \times 1} = 23.41 \text{ mg/L} = 23.4 \,\mu\text{g/ml}$$

[b] Mean of 6 replicate measurements

[c] Mass concentration calculated on weighed amount of sample with expanded uncertainty according to GUM [4]

[d] confidence interval with P = 95 %

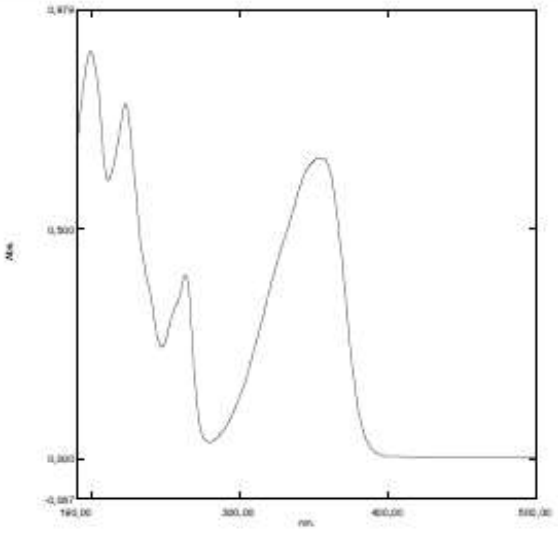


Figure 4: UV absorption spectrum of Aflatoxin B1 sample

5.2. HPLC-DAD

The purity check using LC-DAD of the AFB1 sample showed one main peak. The peak purity of the main signal was examined by diode array spectra of the AFB1 peak and led to the conclusion that this peak consists only of AFB1.

column	Phenomenex Luna C18(2), 250 x 3.0 mm, 5µ
injection volume	100 µL sample
solvent A	water/ acetonitrile/ methanol 57/17/26
oven	30°C
flow rate	0.5 mL / min
DAD settings	365 nm
sample dilution	1:5 with water

	time	area	height
Aflatoxin B1	14.213	6.346	23.318

* Mean of 7 replicate measurements against reference batch, confidence interval with P = 95 %

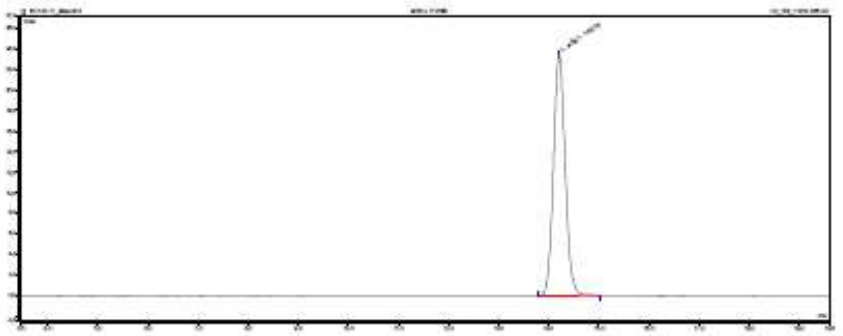


Figure 1: HPLC-UV chromatogram of Aflatoxin B1

6. Further information

The purchaser must determine the suitability of this product for its particular use. LGC Standards GmbH makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by LGC Standards GmbH. We do not guarantee that the product can be used for a special application.

approved for release by: *Laurence Treccani-Chinelli, Global Supply Chain Manager - LGC Standards* *date:* 11.07.2017

This document has been computer generated and is valid without a signature.

Safety data sheet

according to 1907/2006/EC, Article 31

Printing date 26.06.2020 *Version number 2* *Revision: 26.06.2020*

SECTION 1: Identification of the substance/mixture and of the company/undertaking

· **1.1 Product identifier**

· **Product name:** **Aflatoxin B1**

· **Part number:** DRE-C10047100
· **CAS Number:**
1162-65-8
· **EC number:**
214-603-3
· **1.2 Relevant identified uses of the substance or mixture and uses advised against**
No further relevant information available.
· **Application of the substance / the mixture** Reference material for laboratory use only

· **1.3 Details of the supplier of the safety data sheet**
· **Manufacturer/Supplier:**
LGC Limited
Queens Road
Teddington
Middlesex TW11 0LY
UNITED KINGDOM

Tel :+44 (0) 20 8943 7000
Fax :+44 (0) 20 8943 2767
eMail : gb@lgcstandards.com
Web : www.lgcstandards.com

· **Further information obtainable from:**
Product safety department
eMail : sds-request@lgcgroup.com
· **1.4 Emergency telephone number:**
For Hazardous Materials or Dangerous Goods Incident
Spill, Leak, Fire Exposure, or Accident
Call CHEMTREC:
USA & Canada 1-800-424-9300

What information should accompany a CRM

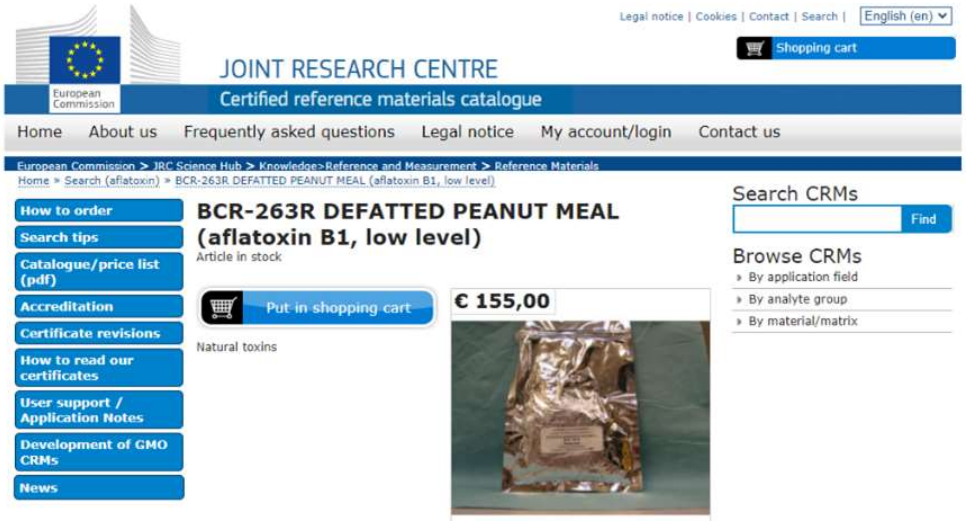
- As for RM plus **Traceability**
- Example: JRC CRM for Aflatoxin B_1 (17.1 ± 2.4 µg/kg) and sum of B_1 B_2 G_1 G_2 (23.7 ± 2.5 µg/kg)

Downloads for this reference material

Certificate BCR-263R certificate.pdf
Certification report BCR-263R_report.pdf
Origin certificate BCR-263R_origin.pdf

Product information

CRM code	BCR-263R
Description on the invoice	PEANUT MEAL
Sales unit	sachet
Net mass	100.000
Gross mass	340.000
Mass unit	Gram (g)
Storage temperature	-20 °C



https://crm.jrc.ec.europa.eu/p/q/aflatoxin/BCR-263R-DEFATTED-PEANUT-MEAL-aflatoxin-B1-low-level/BCR-263R

JRC CRM for Aflatoxin

- **Certificate of Analysis (3pp)**
  - Gives the certified (and indicative) values, expanded measurement uncertainty & k
  - Confirms the certified values are traceable to the SI
  - Outlines the methods of analysis
  - Lists the labs (n = 8) who carried out the analysis, all accredited to ISO 17025 for the method
  - Instructions for use and storage information
- **Certification Report (41pp)**
  - See next slide
- **Origin Certificate (1 page)**
  - Who prepared the material
  - Originated from Chinese peanuts
  - Free from exposure to exotic disease

EUROPEAN COMMISSION

JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements

CERTIFIED REFERENCE MATERIAL
BCR® – 263R

CERTIFICATE OF ANALYSIS

DEFATTED PEANUT MEAL		
	Mass Fraction	
	Certified value [1] [µg/kg]	Uncertainty [2] [µg/kg]
Aflatoxin B_1	17.1	2.4
Aflatoxin B_2	3.0	0.4
Aflatoxin G_1	3.0	0.5

1) These values are the mass fractions based on the unweighted means of accepted sets of results. The values are traceable to the SI. The aflatoxin mass fractions as stated are defined by the employed reversed phase liquid chromatography methods with post column bromination, fluorescence detection and immunoaffinity clean up.

2) Expanded uncertainty with a coverage factor of $k = 2$, according to the Guide for the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 10 g.

Geel, May 2008
Latest revision: September 2011

Signed:

Prof. Dr. Hendrik Emons
European Commission
Joint Research Centre
Institute for Reference Materials and Measurements
Retieseweg 111
B-2440 Geel, Belgium

JRC

JRC CRM for Aflatoxin – Certification Report

- Glossary, Introduction, Participants
- Processing, Additional Characterisation (water content, water activity, particle size)
- Homogeneity and Stability studies
- Certification (Study Design, Results & Technical Evaluation, Certified Values & MU,
- Metrological Traceability
- Commutability
- Instructions for use, Storage conditions, Safety precautions,
- Use of the certified value
- Acknowledgements , References
- Annex A. Homogeneity data
- Annex B. Stability data
- Annex C. Certification measurements

Aflatoxins mass fractions are defined by the employed methods. As three different solvents and extraction techniques have been used independence from the extraction method can be assumed. The certified values are traceable via the common calibrants used, which are certified for aflatoxins in acetonitrile, traceable to SI due to the gravimetric preparation employed.

BCR-263R is prepared from naturally contaminated material … no reason to assume it would behave differently from natural samples with similar particle size.

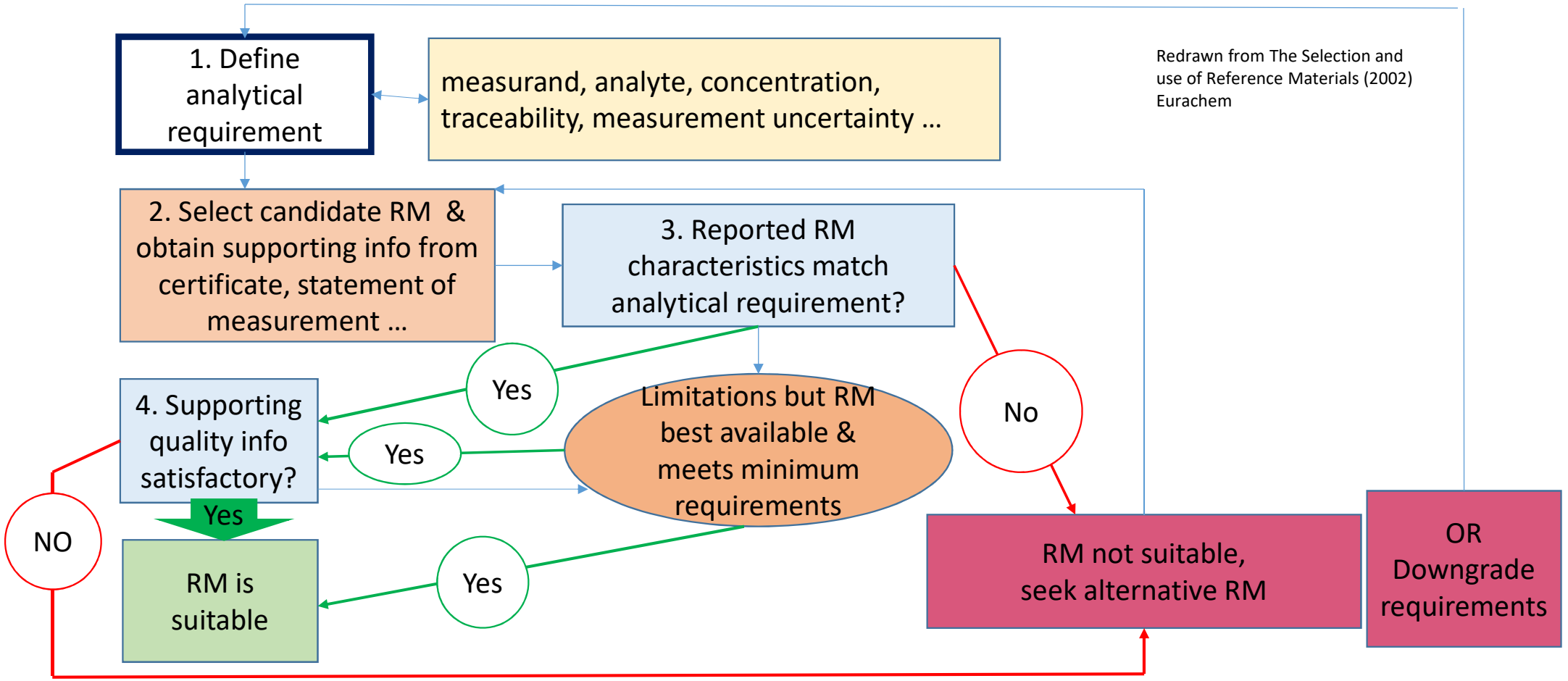
file:///C:/Users/M.Walker/Downloads/BCR-263R_report.pdf

Selection of RM and CRM

The Selection and use of Reference Materials (2002)
https://www.eurachem.org/index.php/publications/guides/usingrm

- Several hundred organisations produce tens of thousands of reference materials worldwide
- Not all materials that are used as reference materials are described as such.
  - Commercially available chemicals of varying purity, commercial matrix materials and products from research programs are often used as standards or reference materials
- Responsibility of the user to assess the information available
- The factors to consider include:
  - Measurand including analyte, Measurement range (concentration), Measurement uncertainty
  - Matrix match and potential interferences, Sample size
  - Homogeneity and stability
  - Value assignment procedures (measurement and statistical)
  - former experiences with the supplier
  - general impression about the material and packaging
  - level of information given in the certificate
  - time between certificate issue and delivery time of RM at the laboratory

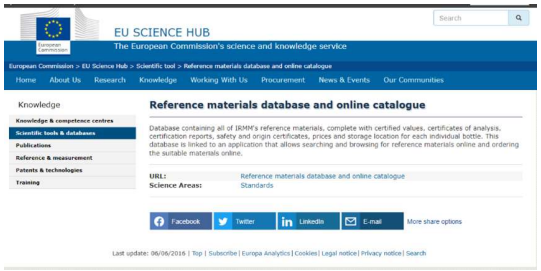
Simple flow chart to assess RM

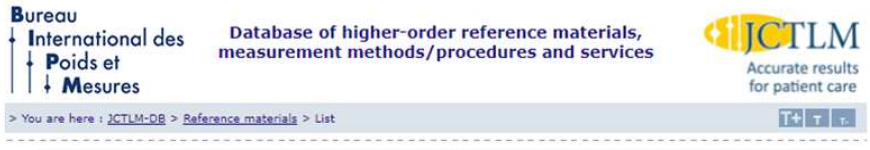


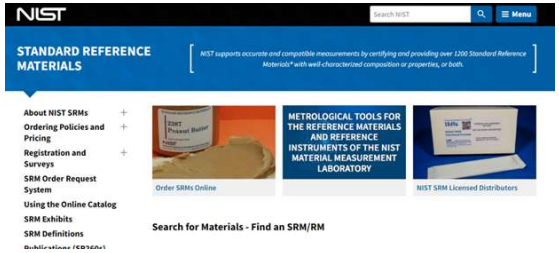
Databases

- https://ec.europa.eu/jrc/en/scientific-tool/reference-materials-database-and-online-catalogue
- https://www.bipm.org/jctlm/viewResults.do?type=isRM&searchString=potassium&searchStringIUPAC=&searchStringMixed=&analyteCategory=&matrixCategory=&sortBy=Analyte_Name&status=0&id=C14RM11R&x=79&y=3
- https://rrr.bam.de/RRR/Navigation/EN/Reference-Materials/COMAR/comar.html
- https://www.nist.gov/srm
- https://en.nim.ac.cn/taxonomy/term/122
- https://www.lgcstandards.com/GB/en?gclid=CjwKCAiA-9uNBhBTEiwAN3IlNBVw_Iou0NP2k_KoDKwK329txM2tHUChHSESyanYgJ7BgHRBvxcIGRoCAhEQAvD_BwE

Check with the NMI or DI in your region







In-house RMs

- High quality RMs are demanding and costly to produce and if materials are available from other sources it is not normally cost effective for laboratories to make their own.
- However should this be necessary, there are some key issues
  - selection of materials
    - appropriateness, native material versus spikes, material preparation …
  - Preparation and packaging (to assure homogeneity, absence of contamination, stability ...),
  - Homogeneity and stability evaluation
  - Certification studies, uncertainty estimation, documentation and QA,
  - Certification approval,
  - Storage, distribution and re-making.

Handling and storage

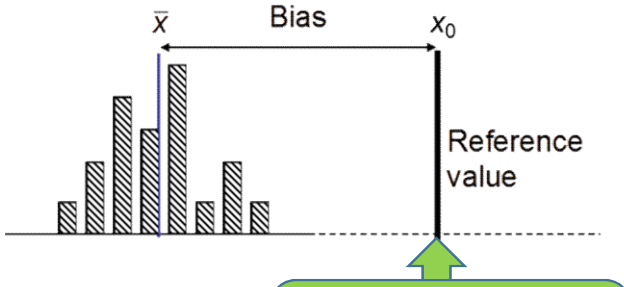
- Short term transportation stability studies include reasonably foreseeable temperature extremes to decide optimum transportation conditions – check these have been observed en route
- Storage conditions are given on the CoA or Statement of Measurement, e.g.
  - **INSTRUCTIONS FOR USE** Samples should be allowed to warm to ambient temperature (e.g. overnight) before opening to avoid water condensation. The contents should be thoroughly mixed before sub-samples are taken. The material should be weighed out immediately after opening the [sachets / vial/ …] and the mass fraction of the measurand calculated based on this mass.
  - **STORAGE** The materials should be stored at or below - 20 °C. However, the [RM Producer] cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples
- Consider if the RM can be mixed without opening or inserting anything (spatula/glass rod) e.g. by shaking the unopened vial, tumble mixing or palpating the sachet, and then transfer a sub-portion to a clean container for manipulation, quickly re-closing the original container and returning it immediately to the recommended storage conditions
- Always mark the date of opening (and sign it) on the container

RM use in method validation

- **Method Validation and Measurement Uncertainty**

Bias (accuracy, 'trueness')

- Appropriate RM / CRM can provide valuable information
- Replicate measurement of the RM, covering the full range of variables permitted by the method being validated can be used to estimate the uncertainty associated with any bias, which should normally be corrected for
- The uncertainty associated with an RM should be no greater than one third of that of the sample measurement
- RMs can be used for training, for checking infrequently used methods and for trouble shooting when unexpected results are obtained



Provided by RM or CRM

Correct use a valid method depends on operator skill , suitability of equipment, reagents and standards.

RM use in calibration

- A (solution of) a pure substance RM is used for calibration
- The purity uncertainty contributes to the total uncertainty of the measurement.
  - For example, an RM certified as 99.9% pure, with an expanded uncertainty U (k=2) of 0.1% will contribute an uncertainty component of 0.1% to the overall measurement uncertainty budget. In the case of trace analysis, this level of uncertainty will rarely be important but for assay work, it can be expected to be significant.
- For matrix RMs a close matrix match is required, the analyte form must be the same in the samples and RMs, and the analytical concentrations of the RMs must span that of the samples
- Other components of the test method, such as sample digestion, separation and derivatisation are, of course, not covered and loss of analyte, contamination and interferences and their associated uncertainties must be addressed as part of the validation of the method.
- Some other methods, such as XRF, use matrix RMs for calibration of the complete analytical process.

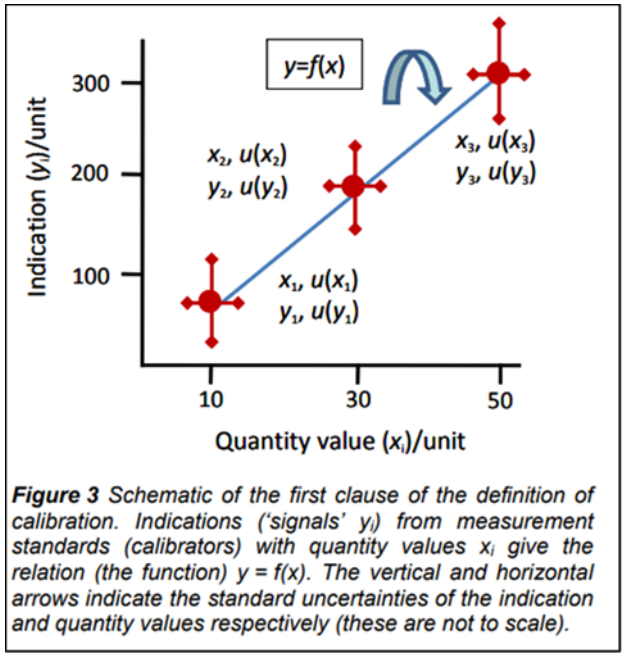
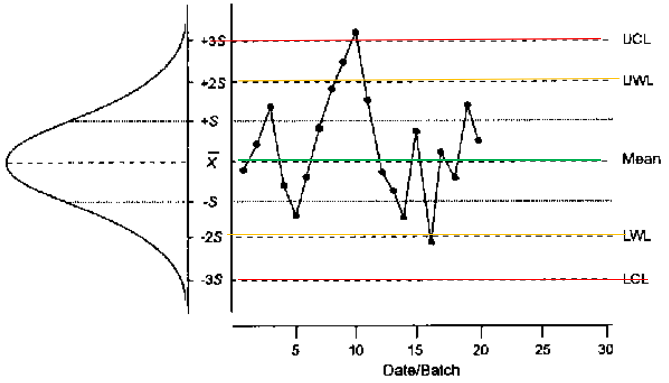


Figure 3 *Schematic of the first clause of the definition of calibration. Indications ('signals' y_i) from measurement standards (calibrators) with quantity values x_i give the relation (the function) $y = f(x)$. The vertical and horizontal arrows indicate the standard uncertainties of the indication and quantity values respectively (these are not to scale).*

RM use in analytical QC

RMs may be used for

- Training, for checking infrequently used methods and for trouble shooting when unexpected results are obtained
- Control charts
  - Either as RM or to validate an in-house QC material
- LoD/LoQ
  - Replicate analyses of low level RM or blank RM
- Instrumental drift assessment
- Ruggedness
- Recovery
- Working and linear ranges



FAO, Internal QC of data
https://www.fao.org/3/w7295e/w7295e0a.htm

Comparing your result with the certified value

The method of Linsinger (Linsinger, T., 2010)

- JRC Application Note (references are to CRM but may apply to RM given sufficient information on the RM)
- Calculate the absolute difference between mean measured value and the certified value (Δ_m).

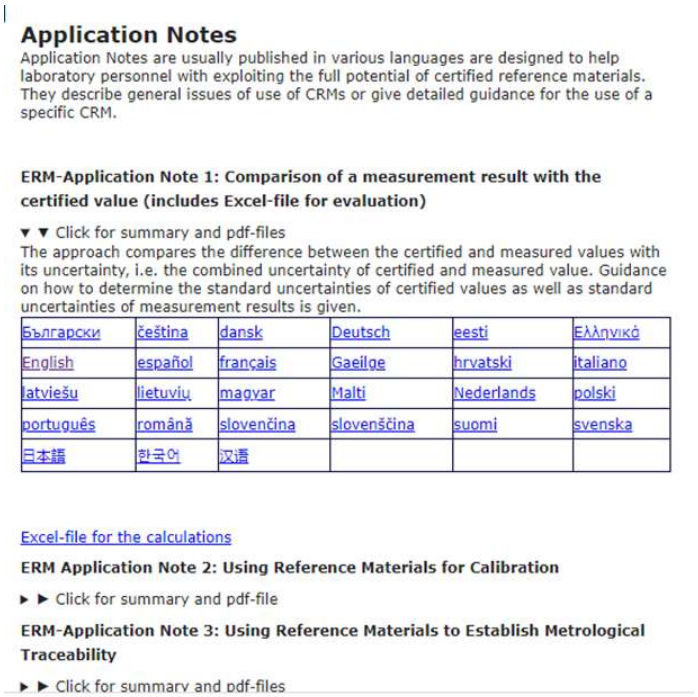
$$\Delta_m = \left| C_m - C_{CRM} \right|$$

- C_m: mean measured value of RM in your lab, C_{CRM}: certified value of CRM
- Calculate the uncertainty $u_\Delta of$ Δm

$$u_\Delta = \sqrt{u^2{}_M + u^2_{CRM}}$$

- Calculate the combined expanded uncertainty ($U\Delta$) using a coverage factor k = 2)
- If $\Delta m \leq U\Delta$ then there is no significant difference between the measurement result and the certified value, at a confidence level of about 95 %
- Linsinger helpfully gives a worked example and it is straightforward to set up a simple spreadsheet to do the calculations

https://crm.jrc.ec.europa.eu/e/132/User-support-Application-Notes



Application Notes

Application Notes are usually published in various languages are designed to help laboratory personnel with exploiting the full potential of certified reference materials. They describe general issues of use of CRMs or give detailed guidance for the use of a specific CRM.

ERM-Application Note 1: Comparison of a measurement result with the certified value (includes Excel-file for evaluation)

▼ ▼ Click for summary and pdf-files

The approach compares the difference between the certified and measured values with its uncertainty, i.e. the combined uncertainty of certified and measured value. Guidance on how to determine the standard uncertainties of certified values as well as standard uncertainties of measurement results is given.

Български	čeština	dansk	Deutsch	eesti	Ελληνικά
English	español	français	Gaeilge	hrvatski	italiano
latviešu	lietuvių	magyar	Malti	Nederlands	polski
português	română	slovenčina	slovenščina	suomi	svenska
日本語	한국어	汉语			

Excel-file for the calculations

ERM Application Note 2: Using Reference Materials for Calibration

▸ ▸ Click for summary and pdf-file

ERM-Application Note 3: Using Reference Materials to Establish Metrological Traceability

▸ ▸ Click for summary and pdf-files

Sources of further information, training and guidance

- International vocabulary of metrology — Basic and general concepts and associated terms (VIM) / *Vocabulaire international de métrologie — Concepts fondamentaux et généraux et termes associés (VIM)* https://www.iso.org/sites/JCGM/VIM/JCGM_200e.html
- Vicki Barwick & Steve Wood, 2010, Achieving metrological traceability in chemical and bioanalytical measurement, Tutorial Review, *J Analyt Atomic Spect,* 25, 785-799.
- BIPM, through the Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM) https://www.bipm.org/en/committees/cc/ccqm
- ISO 17034 :2016 General requirements for the competence of reference material producers
- ISO/Guide 31:2015 Reference materials — Contents of certificates, labels and accompanying documentation
- ISO GUIDE 35:2017 Reference materials — Guidance for characterization and assessment of homogeneity and stability
- The Selection and use of Reference Materials (2002) https://www.eurachem.org/index.php/publications/guides/usingrm and all other guidance at https://www.eurachem.org/index.php/publications/guides
- JRC Catalogue, 'How to read our certificates', User support / application notes and other guidance at https://crm.jrc.ec.europa.eu/e/4/How-to-order
- ENFSI, Guidelines on the use of reference materials in forensic drug analysis
- AMC Technical Brief, 2008, Test for 'sufficient homogeneity' in a reference material https://www.rsc.org/images/homogeneity-test-technical-brief-17A_tcm18-214886.pdf
- See also other Technical Briefs at https://www.rsc.org/membership-and-community/connect-with-others/through-interests/divisions/analytical/amc/technical-briefs/

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