doi:10.2533/chimia.2009.629 Chimia 63 (2009) 629–631

The Role of Reference Materials in Chemical Metrology

Thomas P. J. Linsinger* and Hendrik Emons

Abstract: Reference materials play an important role in chemical metrology. Besides ensuring traceability of measurement results, they contribute to the uncertainty budget of measurement results. This paper discusses the main uses of reference materials (certified or not) and describes their impact on establishing traceability and uncertainty budgets.

Keywords: CRM · Metrology · Reference materials

1. Introduction

Using the 'General requirements for the competence of testing and calibration laboratories' (ISO 17025) as the measure for good analytical practice, the important role of reference materials in chemical metrology becomes apparent.[1] Section 5.6 on traceability devotes a complete sub-section to reference materials. However, discussions with laboratories, users of measurement data and sometimes reference material producers themselves reveal that there still exists some ambiguity about what is comprised by the term 'reference material' and what they can be used for. This ambiguity is reflected in *e.g.* ISO/DIS 17043 on proficiency testing which describes 'test items', while failing to recognise that these 'test items' are in fact reference materials.[2] ISO Guide 30 contains the following definitions:[3]

Reference material (RM): material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

Certified reference material (CRM): reference material characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

The generic term 'reference material' therefore comprises non-certified as well as certified materials, for example quality control materials used for control charts, samples for proficiency tests, as well as calibration standards, reference substances from Pharmacopoeias and matrix CRMs. The distinction between certified and non-certified reference materials is only easy at the extremes: materials used for control charts generally do not have independently assigned values, whereas matrix CRMs, standards and Pharmacopoeia substances clearly come with certificates. The issue is ambiguous for proficiency test materials: Obviously, no certificate is delivered with the samples, but the very nature of a proficiency test requires property value assignment. Consequently, some proficiency testing schemes sell leftover materials as certified reference materials.

Especially in clinical chemistry a distinction between 'primary' and 'secondary' reference materials is made, with the implication that the former have a higher metrological status than the latter. Unless the 'primary reference material' actually defines the measurand, this distinction is not useful – the guiding principle is the uncertainty of the assigned value, rather than the mode of value assignment.

2. Uses of Reference Materials

In a very general sense, every measurement process can be broken down into sampling, sample preparation, quantification and data evaluation (Fig.). Different types of reference materials can be used at the different stages to ensure analytical quality:

- *Sampling*: Although there have been suggestions of denominating 'reference plots' for soil sampling, external quality control of the sampling process is usually limited to dedicated intercomparisons on sampling. This situation is due to the large amount of material necessary to allow repeated sampling, which makes production of several 'reference sampling sites' unfeasible from a logistical point of view.
- *Sample preparation*: Matrix materials resembling as closely as possible the real sample can be fed into the normal analytical process. The result of these measurements is then compared with the assigned value. Here two possibilities exist: i) An independently assigned value is available (CRMs, proficiency tests): Agreement with the assigned value within the respective uncertainties demonstrates absence of any significant bias of the result. ii) The assigned value is laboratory specific (quality control charts): Agreement of the result with the assigned value demonstrates that the method was performed as specified and that no gross errors occurred.
- *Calibration*: In most cases, pure standards are used to calibrate analytical systems to establish analyte-response curves. Naturally, in order to do so, values must be assigned to the reference materials in question. Therefore, all calibration standards must fulfil the technical requirements of certified reference materials, even if no formal certificate in compliance with ISO Guide 31 is issued.[4,5]
- *Evaluation*: In theory, reference datasets could be available to check the correct evaluation of results. While this principle is widespread in computer science to test algorithms, no such datasets are available for chemical analysis, presumably as it is seen more useful to check the complete analytical system *via* matrix CRMs. In addition, slight differences in analytical systems will render reference datasets useless for most laboratories.

*Correspondence: Dr. T. P. J. Linsinger
European Commission
Joint Research Centre
Institute for Reference Materials and Measurements (IRMM)
Retieseweg 111
B-2440 Geel, Belgium
Tel.: +32 14 571 956
Fax: +32 14 571 548
E-mail: thomas.linsinger@ec.europa.eu

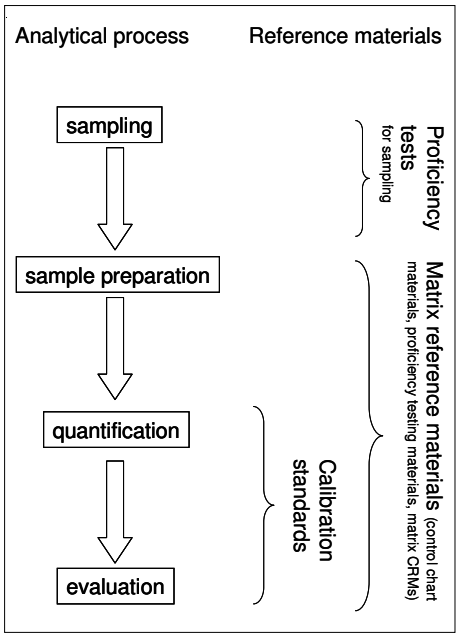

Fig. Schematic description of the analytical process and the reference materials involved.

This brief overview shows that it is the application that determines the requirements, not the name, as also discussed previously:[6]

- *Demonstration of stability of the analytical process*: The material in use must be homogeneous and stable, as subsamples of the same material are used in each measurement series. No absolute assigned value is necessary, as only a relative assessment is made. Laboratory internal quality control materials are usually used for this purpose.
- *Demonstration precision and absence of bias*: Materials must have a reliable assigned value. They must only be stable for the time of the measurement. Long-term stability and between-bottle variation is important if batches of materials are produced. Certified matrix reference materials and proficiency test materials are used in this case.
- *Calibration of equipment*: Reliable assigned values must be available. Uncertainties must be as small as possible, as any uncertainty of the assigned values is directly taken over in the uncertainty of the measurement results. For this reason, pure substance materials are preferred over matrix materials, due to the lower uncertainty of the assigned values. Calibration standards are prime examples for this purpose.

3. Reference Materials and Chemical Metrology

Reference materials have a key role in the two main concepts of metrology, namely uncertainty estimation as well as traceability. The contribution of reference materials to establishing traceability are also summarised in the Fig.

3.1 *Traceability*

In some cases, reference materials are the ultimate reference for chemical measurements. Such 'artefact' defined properties include chemical shift (versus a reference material), vitamins stated in international units as well as several primary preparations of World Health Organization reference materials. While defining units *via* artefacts has severe drawbacks like confirmation of stability and replacement of batches, it is in some cases the only feasible way.

More often, however, reference materials are used to establish calibration curves, which are then used to quantify the analyte present in the sample. These calibrants must have a clearly defined and well-established purity. There is a consensus among major reference material producers that 'well established' for organic substances includes testing for residual solvents, potential inorganic impurities, as well as testing for other organic impurities, preferably by several methods. Less stringent criteria apply for internal standards, as long as the same batch and the same amount is used for calibration standards and samples.

By the same token, all physical input factors (volumes, temperatures, masses *etc.*) must be calibrated by reliable reference materials.

Certified reference materials have an especially important role in ensuring traceability of results of method-defined properties like enzymatic activity, dietary fibre or microbiological testing. Absence of ill-defined interferences or inhibitions can only be verified by analysing CRMs.

Finally, reference materials play an important role in method validation. Homogeneous and stable (*i.e.* reference) materials are needed to establish the precision of a method. After potential biases are identified *via* method optimisation, CRMs or participation in proficiency tests are used to confirm that indeed all biases have been identified.

3.2 *Uncertainty Estimation*

Data from reference materials contribute at several steps to the uncertainty budgets of chemical measurements.

The uncertainty of the assigned values of calibrants contribute directly to the uncertainty of measurement results. Calibrants without stated uncertainty do not allow complete uncertainty budgets to be drawn up, which renders them in practice useless. It is for this reason that every calibrant has to fulfil all requirements of CRMs, even if the producer may shy away from using this term.

If uncertainty budgets are established bottom-up, certified reference materials or intercomparisons are used to confirm the completeness of the uncertainty budgets. In this case, the uncertainties of the certified values are used for assessing agreement/disagreement, but are not a part of the uncertainty budget.[7]

In many cases, uncertainties are derived from intra- or interlaboratory validation studies. Frequently, recovery factors of extraction steps are derived from results on CRMs or proficiency tests. The uncertainties of the assigned values are a part of the uncertainty of trueness in such approaches.[8]

4. Sources of RMs and Quality Assessment of RMs

While the websites of reference material producers provide information on their RMs and user support, distributers have frequently taken over the task of actual delivery of materials. Catalogues of distributors comprise RMs of many producers, thus facilitating finding the most suitable material. Table 1 summarises the websites of the main producers of reference materials.

However, with this easier access, and increased choice, the problem which material to choose arises. As the materials shall ensure analytical quality and traceability of measurement results, quality criteria must be the guiding selection principle. In short, users should ensure that the RMs have been produced in line with the 'General criteria for the competence of reference material producers' (ISO Guide 34).[9] Ideally, the reference material producer is accredited to ISO Guide 34 by a signatory of the ILAC mutual recognition arrangement of accreditation bodies. Until 2004, such accreditation was restricted to producers of the USA and the Asian/Pacific region, but has gained momentum also in Europe since then. Following the lead of IRMM, which was the first European organisation to be granted accreditation to ISO Guide 34 by the Belgian accreditation body BELAC, also the accreditation bodies of Switzerland, the United Kingdom, Germany and other European countries offer now accreditation of RM producers. Where no formal accreditation is available, enrolment in voluntary assessment schemes or self-declaration can enhance trust in the assigned values. A careful investigation whether certificates issued comply with the requirements of ISO Guide 31, whether they contain clear traceability statements and measurement uncertainties in line with the 'Guide to the expression of uncertainty in measurements' can complement such self-declarations by producers. A potential 'metrology' checklist is given in Table 2.

Table 1. Main public producers of matrix materials. Also three databases (COMAR, VIRM, ERM) comprising RMs from various producers are listed

Institute	Website
Bundesanstalt für Materialforschung und -prüfung (BAM) (DE)	*http://www.bam.de/en/fachthemen/referenzmaterialien/index.htm*
Institute for Reference Materials and Measurements (IRMM), Joint Research Centre, European Commission (EU)	*https://irmm.jrc.ec.europa.eu/html/reference_materials_catalogue/index.htm*
International Atomic Energy Agency (IAEA) (UN)	*http://www.iaea.org/programmes/aqcs/database/database_search_start.htm*
Korean Research Institute of Standards and Science (KRISS) (KR)	*http://crm.kriss.re.kr/english/index.jsp*
LGC Standards (UK)	*http://www.lgcstandards.com/home/home_en.aspx*
National Institute of Metrology (NIM) (CN)	*http://en.nim.ac.cn/*
National Institutes of Standards and Technology (NIST), USA	*http://ts.nist.gov/measurementservices/referencematerials/index.cfm*
National Metrology Institute of Japan (NMIJ) (JP)	*http://www.nmij.jp/english/service/C/*
National Research Council of Canada (NRCC) (CA)	*http://www.nrc-cnrc.gc.ca/eng/services/inms/reference-materials.html*
COMAR database (largest compendium of reference materials)	*www.comar.bam.de*
ERM-initiative	*www.erm-crm.org*
Virtual institute for reference materials (also a database for reference materials)	*http://www.virm.net*

Table 2. Potential checklist to assess metrological reliability of RMs

i) *Metrologically critical issues*
- Have all measurands been tested for homogeneity?
- Have all measurands been tested for stability?
- Are values assigned? if yes,
  - Is the measurand clearly defined?
  - Are the assigned values derived from a metrologically valid procedure?
  - Are the assigned values clearly stated on a document (certificate *etc.*)? Is this document in line with ISO Guide 31? [5]
  - Do the assigned values have uncertainties?
  - Do the assigned uncertainties comprise contributions of homogeneity, stability and characterisation?
  - Is the coverage factor for the uncertainties clearly stated?
  - Is the traceability of the assigned values clearly defined?

ii) *Issues critical for practical use*
- Are instructions for use given?
- Is a minimum sample intake stated?
- Is the shelf-life clearly defined?

iii) *Issues ensuring consistent quality*
- Does the producer have a quality system, so that the basis for the above statements is documented and can be traced back?

Finally, if none of the above is available, one will have to resort to the track record of a producer, which, without support of a quality system, is not necessarily a good predictor for future performance.

Attempts have been made recently to quantify reliability of reference materials: Various CRMs were measured and the results were compared to the assigned values by Lu *et al.*[10] Although this approach seems elegant, it is very difficult to implement in practice. The main difficulty is that this approach implies putting more trust on one's measurements than into the assigned values. A brave assumption, seeing the efforts usually made by reference material producers. A further difficulty is that testing the increasing number of reference materials exceeds the capacities of any laboratory by far. In practice, users will therefore have to rely on quality assessment schemes to evaluate the reliability of the assigned values.

5. Future Challenges and Outlook

Production of reference materials and value assignment of certified reference materials has been consolidated with the revision of ISO Guide 35 in 2005 (CRMs) and the upcoming revised version of ISO Guide 34 (all RMs). However, while ISO Guide 34 is equally usable for preparing materials for qualitative analysis (identity *etc.*), adaptations of the statistical approaches for value assignment described in ISO Guide 35 will be needed for these materials. While the principles to be applied are the same (quantification of homogeneity, stability, uncertainty of characterisation), more work is needed on how 'identity' can be heterogeneous.

Work is ongoing in the ISO Committee on Reference Materials (ISO/REMCO) to provide guidance also on these kinds of reference materials.

Received: July 28, 2009

[1] ISO 17025, 'General competence for the competence of testing and calibration laboratories', ISO, Geneva, **2005**.
[2] ISO Guide 30, 'Terms and definitions used in connection with reference materials', ISO, Geneva, **1992**, amended **2008**.
[3] ISO DIS 17043, 'Conformity assessment – General requirements for proficiency testing', ISO, Geneva, **2009**.
[4] ISO Guide 35, 'Reference materials – General and statistical principles for certification', ISO Geneva, **2005**.
[5] ISO Guide 31, 'Reference materials – Contents of certificates and labels', ISO, Geneva, **2000**.
[6] H. Emons, T. P. J. Linsinger, B. M. Gawlik, *Trends Anal. Chem.* **2004**, *23*, 1.
[7] W. Hässelbarth, *Accred. Qual. Assur.* **2004**, *9*, 509.
[8] T. P. J. Linsinger, *Trends Anal. Chem.* **2008**, *27*, 916.
[9] ISO Guide 34, 'General requirements for the competence of reference material producers', ISO Geneva, **2000**.
[10] X. Lu, H. Li, F. Meng, J. Ji, *Accred. Qual. Assur.* **2009**, *14*, 329.