# TDRM Guidelines:

# Applications and Resources for Use of Reference Materials

AOAC INTERNATIONAL
Technical Division on Reference Materials

# INTRODUCTION

**How reliable are your measurements?**

*How close is close enough?*

**Definitions** – *a good place to start*

**Overview** – *what is the function of a reference material and what do the results mean?*

***Additional Resources***

# A Reliable Measurement is Worth a Thousand Opinions

Throughout life we learn that we can measure things – a teaspoon of sugar, a cup of milk, the length of a piece of lumber, or the distance we have driven. It is easy to view these numbers as solid, non-arbitrary facts.

In development of analytical processes in the laboratory, we realize that measurements are only as good as the standards by which the measurement procedures are validated and the tools calibrated. We view our numbers as projected values with associated uncertainties and describe those values using the correct analytical terminology to provide meaningful results.

Reliable data in a measurement process begins before the sample enters the laboratory. How accurately the sample represents its source, how the sample is handled during transfers and whether it is properly stored directly affect the value of the results.

# How well do your measurements measure up?

After the sample enters the domain of the laboratory, the reliability of the measurement depends upon the quality control process. This process must be comprehensive of all aspects of laboratory operations, and will include most or all of the following elements:

- *recordkeeping*
- *sample handling and storage*
- *equipment maintenance and calibration*
- *software and hardware performance validation*
- *sound method validation*
- *method performance verification*
- *employee training verification*
- *systematic assessment of the quality of analytical results*
- *participation in proficiency test programs*
- *accreditation or certification*
- *internal and external reviews*

# How well do your measurements measure up?

Internally, reference materials support this process at multiple points. Typical applications of certified reference materials include:

- method validation
- method performance verification
- verification of equipment calibration
- establishing values for in-house reference materials

Reference materials are particularly useful for systematic assessment of the quality of analytical results. Certified reference materials may be used anywhere that a quality control material is used, however a quality control material is a practical, cost-effective alternative where repetitive testing of the same material is required.

*There are some specific examples where certified reference materials may be used as calibrants, but a different CRM must be used for quality control purposes, i.e. calibration verification.

NOTE: "Reference materials" is a generic term. Reference materials may be pure substances or matrix materials, and these may be certified or non-certified. Also, data provided for CRMs may include analyte values that are not certified. The different types of reference materials fit different roles in a measurement process. For purposes of this document, non-certified matrix reference materials will be referred to as quality control materials. These are sometimes referred to as "in-house" reference materials or "QC" or "QA" materials. See also: The 'RM family'—Identification of all of its members

# How well do your measurements measure up?

The use of reference materials should be seen as integral to the process of method development, validation, and performance evaluation. Reference materials are not the only component of a quality system, but correct use of reference materials is essential to laboratory operations. A "perfect" result can occur by chance, but **consistent reliability** is only achieved through *systematic quality management*.

The purpose of this document is to provide reference information and examples of how different types of reference materials can be applied to different aspects of analytical quality control.

# Definition: Reference Material

**OPTION 1**: Material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties

Note 1 – Examination of a nominal property provides a nominal property value and associated uncertainty. This uncertainty is not a measurement uncertainty.

Note 2 – Reference materials with or without assigned quantity values can be used for measurement precision control whereas only reference materials with assigned quantity values can be used for calibration or measurement trueness control.

Note 3 – 'Reference material' comprises materials embodying quantities as well as nominal properties.

EXAMPLE 1: Examples of reference materials embodying quantities:

a) water of stated purity, the dynamic viscosity of which is used to calibrate viscometers;
b) human serum without an assigned quantity value for the amount-of-substance concentration of the inherent cholesterol, used only as a measurement precision control material;
c) fish tissue containing a stated mass fraction of a dioxin, used as a calibrator.

EXAMPLE 2: Examples of reference materials embodying nominal properties:

a) colour chart indicating one or more specified colours;
b) DNA compound containing a specified nucleotide sequence; fish tissue containing a stated mass fraction of a dioxin, used as a calibrator.
c) urine containing 19-androstenedione

# Definition: Reference Material

**OPTION 1**, cont.

Note 4 – A reference material is sometimes incorporated into a specially fabricated device.

EXAMPLE 1 Substance of known triple-point in a triple-point cell.

EXAMPLE 2 Glass of known optical density in a transmission filter holder.

EXAMPLE 3 Spheres of uniform size mounted on a microscope slide.

Note 5 – Some reference materials have assigned quantity values that are metrologically traceable to a measurement unit outside a system of units. Such materials include vaccines to which International Units (IU) have been assigned by the World Health Organization.

Note 6 – In a given measurement, a given reference material can only be used for either calibration or quality assurance.

Note 7 – The specifications of a reference material should include its material traceability, indicating its origin and processing (Accred. Qual. Assur.: 2006) [45].

Note 8 – ISO/REMCO has an analogous definition[45] but uses the term "measurement process" to mean 'examination' (ISO 15189:2007, 3.4), which covers both measurement of a quantity and examination of a nominal property.[2]

VIM3, 5.13, TDRM OMA Appendix A 982.35 with revisions

# Definition: Reference Material

**OPTION 2**: 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.

Note 1 – RM is a generic term.

Note 2 – Properties can be quantitative or qualitative (i.e., identity of substances or species)

Note 3 – Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.

Note 4 – A single RM cannot be used for both calibration and validation of results in the same measurement procedure.

Note 5 – VIM has an analogous definition (ISO/IEC Guide 99:2007, 5.13), but restricts the term "measurement" to apply to quantitative values and not to qualitative properties. However, Note 3 of ISO/IEC Guide 99:2007, 5.13, specifically includes the concept of qualitative attributes, called "nominal properties". [3]

TDRM OMA Appendix A 982.35 with revisions

# Definition: Certified Reference Material

**OPTION 1**: 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.

EXAMPLE: Human serum with assigned quantity value for the concentration of cholesterol and associated measurement uncertainty stated in an accompanying certificate, used as a calibrator or measurement trueness control material.

Note 1 – 'Documentation' is given in the form of a 'certificate' (see ISO Guide 31:2000).Uncertainties for such attributes may be expressed as probabilities.

Note 2 – Procedures for the production and certification of certified reference materials are given, e.g. in ISO Guide 34 and ISO Guide 35. *[TDRM Note: see also ISO 17034]*

Note 3 – In this definition, "uncertainty" covers both 'measurement uncertainty' and 'uncertainty associated with the value of a nominal property', such as for identity and sequence.
"Traceability" covers both 'metrological traceability of a quantity value' and 'traceability of a nominal property value'.

Note 4 – Specified quantity values of certified reference materials require metrological traceability with associated measurement uncertainty (Accred. Qual. Assur.: 2006) [45].

Note 5 – ISO/REMCO has an analogous definition (Accred. Qual. Assur.: 2006) [45] but uses the modifiers 'metrological' and 'metrologically' to refer to both quantity and nominal property. [2]

VIM3, 5.14, TDRM OMA Appendix A 982.35 with revisions

# Definition: Certified Reference Material

**OPTION 2**: Reference material, characterized 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.

Note 1 – The concept of value includes qualitative attributes such as identity or sequence.

Uncertainties for such attributes may be expressed as probabilities.

Note 2 – Metrologically valid procedures for the production and certification of reference materials are given in, among others, ISO Guide 34 and 35.

Note 3 – ISO Guide 31 gives guidance on the contents of certificates. [3]

Additional resources for definitions of terms:

Basic and general concepts and associated terms, VIM

Eurachem Guide: Terminology in Analytical Measurement

Accuracy, Trueness, Error, Bias, Precision & Uncertainty, 2015

# Why Reference Materials ?

♦ prerequisites for reliable measurement results

Quality assurance

Sample Collection, Preparation and Storage

Sample preparation

Analyte identification & Quantitative measurement

RM for qualitative analysis

RM for *calibration*

Matrix CRMs

as close as possible to real sample

Evaluation

Assessment

*method validation*

*performance control (methods, labs)*

required RM characteristics

# RM Guidelines Overview

- Primary applications of reference materials include:
  - Method Validation
  - Method Verification
  - Calibration
  - Proof of Method Performance
- Assigning values to other reference materials
- Interpretation of Test Results
- When CRM Needed is Not Available

# Purpose and Applications of RMs in Method Development and Validation

Method development and validation for matrices within the scope of the method is done to characterize attributes such as recovery, selectivity, "trueness" (accuracy, bias), precision (repeatability and reproducibility), uncertainty estimation, ruggedness*, LoQ or LoD and dynamic range. Reference materials should be chosen that are fit-for-purpose. When certified reference materials are available with matrices that match the method scope, a lot of the work involved in method development has already been completed. The property values are traceable through a common reference, as described on the certificate. Reference materials with analyte values in the range of test samples as well as "blank" matrix reference materials, with values below or near detection limits, are needed.

- Selection of identical subsamples for development
- Evaluation of method performance parameters: "Trueness"
- Uncertainty estimation

# Evaluating Method Performance: "Trueness"

Natural matrix CRMs provide the greatest assurance that your method is capable of producing accurate results for that matrix.

CRMs may be used for parameters of method validation and can help to provide traceability of results to the International System of Units (SI). However a well-developed quality control material is typically more practical where repetitive measurements of the same material are needed but will not provide traceability to a more universal reference.

# Assessing Precision or Optimizing a Method

A well-characterized, homogenous and stable quality control material is well-suited for:

- Repetitive testing of matching ("identical") subsamples to assess overall variability arising from the method.
- Assessing the effect of changing a method condition during method optimization. A reference material may be tested under both conditions so that the only difference in results is due to the change in the method parameter.

# Method Verification

Implementation of a validated method in a new laboratory entails less characterization than validation of a completely new method. Certified reference materials with a matrix as close as possible to the sample matrix for which the method is being validated are a reliable tool for characterizing the method attributes. In-house reference materials can be used to establish the precision of the analytical method.

- Evaluation of method performance parameters: "Trueness"
- Uncertainty estimation

# Use of CRMs in Calibration

Some CRMs are produced as "reference solutions" or "reference standards" for the limited purpose of equipment calibration or for assessing equipment calibration. These "pure-substance" or "calibration standard" certified reference materials consist of compounds of stated purities or their solutions. These CRMs may be used:

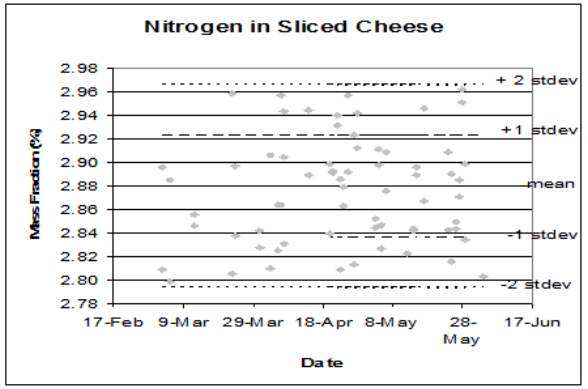
- to establish calibration curves
- as an independent check for equipment calibration, using a different material than the one used for calibration
- to evaluate the suitability of an alternate supplier of calibration standards

Examples of applications of pure-substance reference materials include pharmaceutical reference materials, microbiological reference materials, clinical reference materials and many GMO and allergen reference materials.

In some unusual applications, there are methods for which the only available calibrant is a certified matrix reference material or its solution. One example is in the field of natural toxins where pure toxin CRMs or calibrants are rare. Matrix CRMs may contain a mixture of toxin analogs, for which there are no commercially available pure standards. Calibration using these materials is typically quite expensive for routine work and the values for measurement uncertainty may be large.

# Proof of Method Performance

- **Internal quality control (charting)**
  - Quality control materials analyzed along with test samples are useful for monitoring the performance of your method, identifying problems before results are released for reporting and analyzing and controlling sources of variation.




- **Operator qualification**
  - Quality control materials are good tools for operator training as well as for verification of training. A trained operator should be able to consistently produce results within the established performance characteristics of the method.

- **Equipment calibration verification**
  - Certified reference materials fit for calibration (includes pure substance CRMs), not the same ones used to calibrate the instrument, are a useful independent check on equipment calibration.

For the first two applications, CRMs may be used, but for routine purposes, where applicable, non-certified RMs with in-house established values traceable to CRMs are more economical. See the TDRM booklet, "Development and Use of In-house Reference Materials."

# Assigning Values to Other Reference Materials

- Quality control materials cannot establish the accuracy of a method when used on their own since it is difficult to establish the true value for the analyte in question. If the quality control material is analyzed in conjunction with a suitable CRM, and acceptable data are obtained for the CRM, then the assigned value for the quality control material can be established and used to estimate the accuracy of a method, and to help demonstrate traceability to the CRM if uncertainties are estimated through each step of the process.
  - See the TDRM booklet, "Development and Use of In-house Reference Materials."
  - Comparison of a Measurement Result with the Certified Value, ERM Application Note 1, 2005
  - Traceability – NIST Policy and Supplemental Materials
  - Eurachem Guide: Traceability in Chemical Measurement
- Blank matrix reference materials are useful for ensuring performance at or near the detection limits. These are particularly useful for routine quality control in methods measuring trace levels such as allergens, mycotoxins or drug residues.
- Certifying Nothingness: Blank Reference Materials (requires TDRM membership for access)

# Matrix CRMs

Certified Reference Materials may be natural matrix reference materials, "pure chemical substance" materials (or their solutions) or physical or biological standard materials. The use of matrix CRMs provides comprehensive information about a method system. When matrix CRMs are used for method development, the analyst has an excellent tool to optimize the performance of the method system. Matrix CRMs with analyte levels in a range appropriate to the method and typical samples may be used to assess and establish method performance characteristics, such as accuracy, bias, limit of quantitation and uncertainty estimates as part of the method validation process. This class of RMs is useful for method implementation verification when methods are transferred or as part of routine method performance control along with quality control materials.

Certified reference materials with values near the limit of detection or limit of quantitation are useful for documenting method system performance in that range as part of the method validation process or for routine method performance verification.

Certified natural-matrix reference materials provide the highest degree of assurance of the entire method system being in control. However, both the matrix as well as the analytes within the matrix must be stable within the specified storage conditions. Providing a stable reference material can be challenging where analytes are biologically active, easily oxidized or interactive with other components of the matrix. Certified reference material producers provide assurance of material stability as well as homogeneity.

See ISO REMCO's guide to the guides on requirements for production of CRMs and information on proper use of CRMs. See the TDRM RM Database for assistance locating available reference materials by analyte, matrix or OMA method, with links to certificates for review of details such as shelf life and uncertainty information. NOTE: The manufacturer's certificate is the authoritative resource for all information related to the reference material, and the official method is the only authoritative resource for information related to the method.

# Certificate

Certified Reference Materials are accompanied by a certificate that includes the following key criteria:

- Assigned values with measurement uncertainty and metrological traceability
- Homogeneity
- Stability, with the expiration date for the certificate
- Storage requirements
- Information on intended use
- Identity of matrix

For some reference materials, such as botanical reference materials, the source and authenticity are a very important piece of information that should be included with the certificate.

Even under ideal storage conditions, many analytes have some rate of change. Recertification may be done by the supplier, and a certificate reissued with a different expiration date.

Important details on requirements for the certificate may be found here:

Eurachem Guide: Traceability in Chemical Measurement
Eurachem Guide: Quantifying Uncertainty in Analytical Measurement
Eurachem Guide: Use of Uncertainty Information in Compliance Assessment
Guidance Document on Measurement Uncertainty for GMO Testing Laboratories (JRC)
ISO 17034, General Requirements for the Competency of Reference Material Producers.
ISO Guide 35, Reference Materials, General and Statistical Principles for Certification
ISO Guide 31. Reference Materials – Contents of Certificates and Labels

*For an overview of the organization of the ISO Guides, click here.

# When a CRM is Not Available

There are many analytes for which a CRM with a suitable matrix is not available. This leaves the analyst with few options. For some methods, there may be proficiency testing programs that include a matrix of interest for the analyte. Proficiency testing allows an analyst to compare results with results from other laboratories, which may or may not be using similar methods. Spiking is another technique that may be used. When alternate methods are available, results may be compared between the different methods. These alternatives do not provide the same level of assurance that is gained through the use of a CRM.

- Proficiency Testing Programs
- Spiking
- Quality control materials or "In-house" RMs
- 2009 Symposium Presentations: What Do You Do When No CRMs are Available?
  (requires TDRM membership for access)

# Proficiency Testing

Proficiency testing is useful for training and verification of competence (external benchmarking). It provides information about how your results compare to other laboratories performing the same test - about where your result falls within a central tendency* over a period of time. Careful evaluation of the proficiency study test results can yield additional information, such as multiple distributions, which allows analysts to investigate the cause of differences. For example, different methods may produce somewhat different results, revealing a method bias. Tracking results through a z-score chart is a useful method of monitoring performance over time for trends. Note that unless traceability, measurement uncertainty, stability and homogeneity are determined, no information is available about the accuracy of the results. When no CRMs are available, proficiency testing programs fill an important void.

NOTE: Many times proficiency testing materials have not been evaluated for homogeneity or stability, and in this case do not meet the definition of a certified reference material.

*When results from multiple laboratories and possibly multiple methods, are compared, data may not follow a normal distribution. See the link about Data Distributions below.

**Resources**:

IUPAC: Proficiency Testing for Analytical Chemistry Laboratories, 2006

Eurachem Guide: Selection, Use and Interpretation of Proficiency Testing (PT) Schemes by Laboratories

Representing Data Distributions with Kernel Density Estimates

Ikins, 2007: Challenges Associated With Supporting Constantly Changing Food Contamination Issues With Appropriate Quality Assurance (requires TDRM membership for access)

# Spiking

In the absence of an available CRM, one technique that is sometimes used for assessing performance is the spiking of a matrix reference material with a known quantity of the analyte. When this method is used, it cannot be assumed that the analyte is bound in the same way as it would be in a natural matrix. When a certified blank reference material is available, this is a better choice for constructing a spiked material.

When preparing reference solutions, the pure standards must be completely soluble in the solvent. For insoluble materials in a liquid suspension or for powdered forms of dry materials, validation is required to demonstrate that the analyte is homogeneously distributed and that the response of the detection system to the analyte is not affected by the matrix or preparation technique. When a matrix material is selected for spiking, it should be reasonably characterized to determine that it is sufficiently representative of the matrix of interest. Spiked samples must be carried through all steps of the method. Some analytes are bound in a natural matrix and whether the spiked analyte will behave the same as the analyte in a natural matrix is unknown.

For these applications, instructions for preparing the spikes must be provided from the reference material supplier where certified values, including measurement uncertainty and confirmation of homogeneity, are needed by the end users. Where the spiked material is prepared and stored for any length of time, the stability of the preparation must also be verified.

**Resources:**

TDRM Symposium Speaker Presentations (2009) (requires TDRM membership for access)

# Quality Control Materials

Internally produced matrix reference materials, often referred to as quality control materials or "in-house reference materials", should have a matrix similar to the materials being tested. These materials have been homogenized and sufficient testing has been done to ensure that the storage conditions are appropriate to maintain stability of the material and analyte for the duration of use. Historical information may be available for some quality control materials to confirm the storage conditions required for stability. Certified reference materials have been tested along with the quality control material to ensure that the method is under control while values are being developed for the in-house material.

These materials are primarily useful for continuing method performance control, but may also be used for analyst training or training verification, or to verify implementation when a validated method is transferred to another facility. For training verification, analysis of quality control materials as "blind" samples is useful for establishing that an analyst is capable of producing correct results. Where a method can produce false negatives and false positives, reference materials representing those conditions should be included, with replicates provided in mixed order when those are provided in ordered sets.

Reference materials with analyte levels near the limit of quantitation are useful, along with blanks, for incorporation into routine quality control intervals.

**Resources**:

See the TDRM booklet, "Development and Use of In-house Reference Materials."

ISO Guide 80:2014, Guidance for the in-house preparation of quality control materials (for purchase)

# Estimation of Uncertainty

Every measurement result has an uncertainty, and the contributions toward the overall uncertainty arise from multiple sources. Evaluating the uncertainty for each step of the method is an important component of characterizing the method performance, and is often one of the criteria used in selecting a method for a given application. Estimation of measurement uncertainty can be accomplished by independently assessing the contribution from each source. There are different means of estimating uncertainty and of using uncertainty values in establishing specification standards.

Comprehensive discussion on estimation of uncertainty can be found in the Eurachem Guide to Quantifying Uncertainty in Analytical Measurement. Specific detailed examples for estimating Measurement Uncertainty are listed in OMA Appendix D (*requires AOAC member account*). Another resource for information on uncertainty is NIST Uncertainty of Measurement Results.

**Additional resources**:

Eurachem Guide: Measurement Uncertainty Arising from Sampling

Eurachem Guide: Use of Uncertainty Information in Compliance Assessment

Eurachem Guide: Quantifying Uncertainty in Analytical Measurements

Finnish Environment Institute: MUKit (tool for calculation of uncertainty)

Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories, Nordtest

Guidance Document on Measurement Uncertainty for GMO Testing Laboratories

# Interpretation of Test Results

- NIST Handbook for SRM Users
- Comparison of a Measurement Result with the Certified Value, ERM Application Note 1, 2005

# Additional Resources

- Information on Accreditation
- Resources for Method Validation
- ISO/REMCO System of Guides
- Previous TDRM Symposia & Meeting Speaker Presentations
- The 'RM family'—Identification of all of its members (requires TDRM account)
- Application of Uncertainty to USP's Compendial Reference Standards Program
- Current Eurachem Guides
- Finnish Environment Institute:  MUKit (tool for calculation of uncertainty)

# Accreditation

Both RMs and CRMs play a vital role in the quality management process, and are therefore important in documented practices required for accreditation.

**Resources for ISO 17025 Requirements**:

ISO/IEC 17025: 2015 General Requirements for the Competence of Testing and Calibration Laboratories

ISO 17025: 2015 Revisions Quality Manual Template

AOAC Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food and Pharmaceuticals

Eurachem Guide to Quality in Analytical Chemistry: An Aid to Accreditation

Eurachem Guide: The Selection and Use of Reference Materials

A2LA Training Schedule

ANSI-ASQ National Accreditation Board Training Schedule

# Resources for Method Validation

AOAC Single Lab Validation Guidelines for Botanicals and Dietary Supplements - (Appendix K)

AOAC Standard Method Performance Requirements (Appendix F)

AOAC Performance Tested Methods Program (toward submission to OMA)

Accuracy, Precision and Reliability of Chemical Measurements in Natural Products Research

IUPAC Harmonized Guidelines for Single Lab Validation

Basic and general concepts and associated terms, VIM

Eurachem Guide: The Fitness of Purpose for Analytical Methods

Eurachem Guide: The Selection and Use of Reference Materials

# ISO REMCO System of Guides