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Reference Materials: A review

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Abstract: Several factors have increased the use of reference materials in laboratories. This can be explained by the fact that the reference materials have several roles, namely: the confirmation of the identity of unknown materials and/or the determination of their properties; the calibration of measuring equipment; the validation of methods; the realization of proficiency tests; etc. To be able to produce and use them, a set of standards and guidelines concerning the subject of reference materials has been established. There are several producers of reference materials in many fields, but finding the right choice is sometimes considered difficult given the multitude of materials to be analyzed that do not correspond perfectly to the reference material, especially in the case of matrices. This makes the market always seek new materials. To develop them, five steps are essential: material preparation, homogeneity study, stability study, characterization, and evaluation of measurement uncertainties. These steps are equally important; the fact of highlighting less than one of them will imply a significant decrease in the quality of the reference material developed. This review seeks to furnish the scientific community with a paper elucidating the functions of these materials in research laboratories, the normative references devised to standardize their production and utilization, the factors influencing their production, and the essential steps for their development.

Keywords: Reference material, Homogeneity study, Stability study, Characterization, Evaluation of measurement uncertainties.

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1. INTRODUCTION

Throughout the world, the results of measurements and testing are considered as a base to be able to control industrial production, affirm or disconfirm scientific hypotheses, make diagnoses in the medical field, assess the nature and degree of pollution in the environment, assess the nutritional quality of foods, etc. In this perspective, incorrect measurements and testing can be the source of incorrect decisions and thus generate additional costs or risks. Therefore, accurate measurements are of paramount importance for the functioning of society in all sectors: industry, health, environment, agri-food, etc.

Decision-making is based on the knowledge of information, which in most cases, in the field of research, rests on the results of measurements and testing delivered by the laboratories (1). These results, aiming mainly to answer research questions, are supposed to be of high quality (trueness, precision) to guarantee a relevant interpretation by the researchers. As a result, the prescribers of measurements and testing, who are increasingly aware, have increased their requirements about the reliability of the results provided by the laboratories (2). Measurements and testing results alone, not accompanied by information on their quality and traceability, are no longer considered sufficient and satisfactory; laboratories are obliged to prove the reliability of their measurements and testing at all

stages, from sampling until the delivery of the final report (2).

In this perspective, modalities aiming at ensuring good quality assurance of the measurements and testing and accuracy of the produced data have been established. This includes setting up good laboratory practices and requirements of accreditation organizations (3) as well as participation in interlaboratory comparisons (4) and the use of reference materials. The use of the latter is considered a means for quality control of the results provided since they make it possible to join them to known quantities (4). The certified reference materials are considered an essential link of a metrological traceability chain to ensure that the unity of the result is universal (1). Their use makes the results of measurements and testing comparable to the recognized reference values and those obtained by the international scientific community. Reference materials are of paramount importance when the accuracy and reliability of the measurement and testing results must be guaranteed.

This review aims to provide the scientific community with a paper detailing the roles of these materials in research laboratories, the normative references developed to standardize their production and use, the elements that govern their production, as well as the steps necessary for their development.

2. TERMS AND DEFINITIONS

ISO Guide 30:2015 (5) defines a reference material (RM) and a certified reference material (CRM) as follows :

- Reference material: "material, sufficiently homogeneous and stable concerning one or more specified properties, which has been established to be fit for its intended use in a measurement process". Properties can be quantitative or qualitative (e.g., identity of substances or species). Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.
- Certified reference material: "RM characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability".

That said, the difference between an RM and a CRM is that parameters in the CRM are guaranteed by the producer and are known with great trueness (2).

Different categories of RMs may be encountered: primary RM, secondary RM, and in-house or working RM. By going from first to last, uncertainty increases (6). An RM that is characteristic of a real sample is called matrix RM (examples: soil, drinking water, blood) according to ISO Guide 30:2015 (5). Matrix RM may be obtained directly from biological, environmental, or industrial sources. They may also be prepared by spiking the component(s) of interest into an existing material.

3. NORMATIVE REFERENCES

A panoply of standards and guidelines concerning the topic of RM are established whether by the International Organization for Standardization (ISO) or by other organizations. Below is a non-exhaustive list of these references:

- ISO Guide 30:2015: Reference materials - Selected terms and definitions (5);
- ISO Guide 31:2015: Reference materials - Contents of certificates, labels, and accompanying documentation (7);
- ISO Guide 33:2015: Reference materials - Good practice in using reference materials (8);
- ISO Guide 35:2017: Reference materials - Guidance for characterization and assessment of homogeneity and stability (9);
- ISO 17034:2016: General requirements for the competence of reference material producers (10);
- ISO Guide 80:2014: Guidance for the in-house preparation of quality control materials (QCMs) (11);
- ISO/TR 16476:2016: Reference materials -- Establishing and expressing metrological traceability of quantity values assigned to reference materials (12);
- ISO/TR 79:2015: Reference materials - Examples of reference materials for qualitative properties (13);
- ISO/TR 10989:2009: Reference materials -- Guidance on, and keywords used for, RM categorization (14);
- ISO 11095:1996: Linear calibration using reference materials (15);
- ISO/TR 11773:2013: Global distribution of reference materials (16);
- ISO 15194:2009: In vitro diagnostic medical devices - Measurement of quantities in samples of biological origin - Requirements for certified reference materials and the content of supporting documentation (17);
- ISO/FDIS 33407: Guidance for the production of pure organic substance certified reference materials (under development) (18);
- LAB MR REF 02 established by the Cofrac (Comité français d'accréditation) (19). It's a document concerning the requirements for accreditation of producers of RMs;
- APLAC TC 012: Guidelines for acceptability of chemical reference materials and commercial chemicals for calibration of equipment used in chemical testing (20) established by the APLAC (Asia Pacific Laboratory Accreditation Cooperation).

4. ROLE OF REFERENCE MATERIALS

Several fields, such as chemistry, biology, agri-food, health, environment, etc., use the RMs. These latter

allow the realization of certain practices ensuring the quality of the measurements, namely:

- The determining of the properties of a material (e.g., quantity, hardness, etc.) and/or the confirmation of its identity (1).
- The calibration of certain methods allows to establish the substance signal-quantity interaction (e.g., UV spectroscopy, colorimetry, atomic emissions, chromatography, etc.). The calibration should be done by using the RMs and taking into account the nature of the matrix as much as possible (21). This makes it possible to obtain quantitative results through the comparison of the sample signals and those of the RM(22). The purity of the RM as well as the uncertainty of this purity must be known (22).
- Calibration of measurement equipment (1–3,6,23).
- The development and validation of methods (2,3,6,24) as well as the validation of the modifications applied to the method (3): the validation as one of the requirements of ISO/IEC 17025:2017 (25) should be performed whenever non-standard methods, internally developed methods, or methods outside their intended scope are used. The estimation of the bias (difference between the measured value and the reference value) can be done by using the RM within the limits of the uncertainty of the certified value and the method submitted to validation (1).
- The internal quality control for the monitoring of an analytical process (1–3,6,23,24): the verification of the long-term reproducibility of a method is performed by using the RMs in statistical control systems while creating control charts (a graphical representation of the results obtained from an RM over time, the system is out of control if the upper or lower control limit is exceeded, among others) (3). The RMs thus allow monitoring of the stability of testing (2).
- The comparison of the performances of the methods in the same laboratory (3) by comparing the testing results by using the same RM and several methods.
- The realization of proficiency tests (1,3,6,24): the laboratory that organizes the proficiency test sends an RM to the participating laboratories. The comparison of the testing results of the RM of a laboratory with those of other laboratories using the same technique allows for evaluation of the performance of the laboratory with this technique (3).
- The uncertainty estimation (1–3,6): the realization of the design of experiments by using RMs is necessary for the estimation of all components of the measurement uncertainties and for estimating the uncertainty of measurement by intra-laboratory reproducibility (2). The uncertainty associated with the purity of the RM contributes to the total uncertainty of the measurement (6).
- The traceability of measurement (24): CRMs are used for quantitative measurements, which makes them "traceable" to these CRMs. However, this approach is not always reliable because, in many cases, the CRM does not have the same matrix as the unknown sample (3).
- Materials intended to test the step(s) of a method (3): the method, that contains steps where the sample is physically destroyed (e.g., acid digestion, fusions, dry ash) or the analyte to be determined is extracted from the matrix, can be verified using a CRM with a matrix (matrix CRM) similar to the unknown sample. The use of CRM allows us to demonstrate if there are losses or contaminations by applying the method. The presence of errors in this method can be affirmed or disconfirmed by comparing the certified value of the CRM and the value determined by the laboratory.

5. PRODUCTION OF REFERENCE MATERIALS

5.1. Producers of Reference Materials

To meet the requirements of prescribers for reliable measurements, it is essential to produce RMs in a wide variety of fields. As a result, the RMs market is considered a very active market (1). For the different areas of measurements and testing, there are several RMs and CRM providers. There is a need to ensure the availability of sufficient quantities of these materials as well as their availability in the long term, even if their production is long and expensive (21). The main organizations distributing RMs are (26):

- NIST: National Institute of Standards and Technology, United States;
- NIBSC: National Institute for Biological Standards and Control (WHO international laboratory for biological standards), United Kingdom;
- CENAM: Centro Nacional de Metrología, Mexico;
- IRMM: Institute for Reference Materials and Measurements, European Union;
- LGC: LGC Limited, United Kingdom;
- HECTEF: HECTEF Standard Reference Center Foundation, Japan.

Given the growth of the RM market and the diversity of producers, finding the right choice has become difficult for users. Databases were developed to assist laboratories in finding the CRM that they need by providing information on the available CRM. However, the COMAR (Code of Reference Materials) remains the main source of information on RMs (3). It was produced at the end of 1970 by the LNE (laboratoire national de métrologie et d'essai, France). Formal cooperation between LNE, NPL (National Physical Laboratory, United Kingdom), and BAM (Bundesanstalt für Materialforschung und-Prüfung, Germany) was then established to improve and propagate the database (27). The use of the latter is free.

The COMAR database lists information on thousands of CRMs produced by more than 200 producers in 26 countries.

Figure 1 shows the distribution of the RMs according to their area of use. The biological and clinical domains have the lowest percentage. For the other domains, the distribution is very close, with a dominance of the industrial sector.

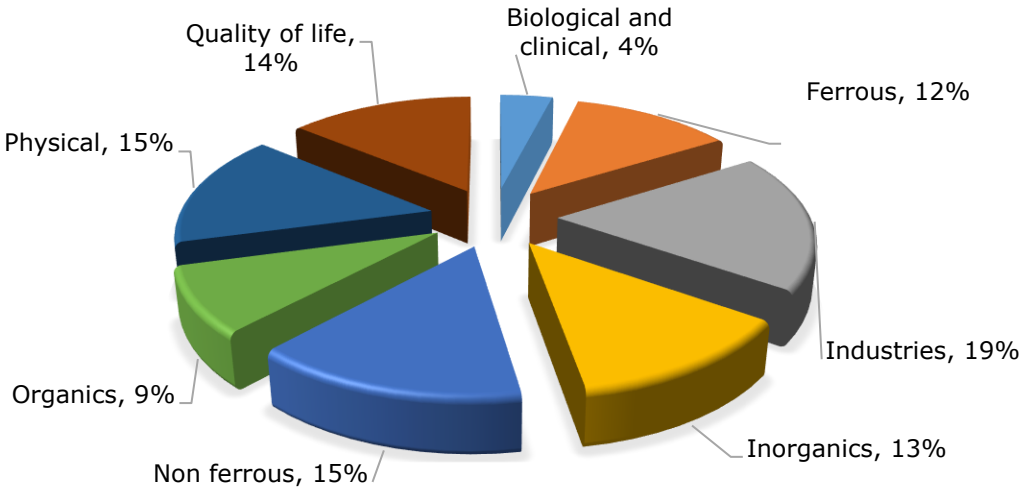

Figure 1: Percentage distribution of CRM by fields of application (27).

Below are some examples of RMs in the environmental, food, and clinical fields (3):

- Environmental analysis: river, lake, estuarine, and marine sediments, as well as fresh-, ground-, and seawater certified for trace elements or trace organics, are some examples of existing CRMs.
- Food analysis: matrices for human nutrition (e.g., meats, cereals, and milk) are usually analyzed for their content of toxic organic (e.g., PAHs, PCBs) or inorganic substances (e.g., heavy metals, major elements), nitrates, pesticides, toxin, and mycotoxins. Nutritional quality and nutritional properties (e.g., fatty acids, sterols, total fat, proteins, carbohydrate oils, fat-soluble vitamins, etc.) are also taken into account during the preparation of certain matrices.
- Clinical analysis: RMs of serum and blood (in lyophilized form) are most often used for the quality control of, for example, toxic elements (e.g., Cd, Pb) or main group elements (e.g., Ca, Li, Mg) and hormones (e.g., cortisol, progesterone). Proteins or enzymes that are partially or highly purified are also used.

Based on the consultation of the COMAR database, a distribution of the origins of the produced RMs is presented in Figure 2.

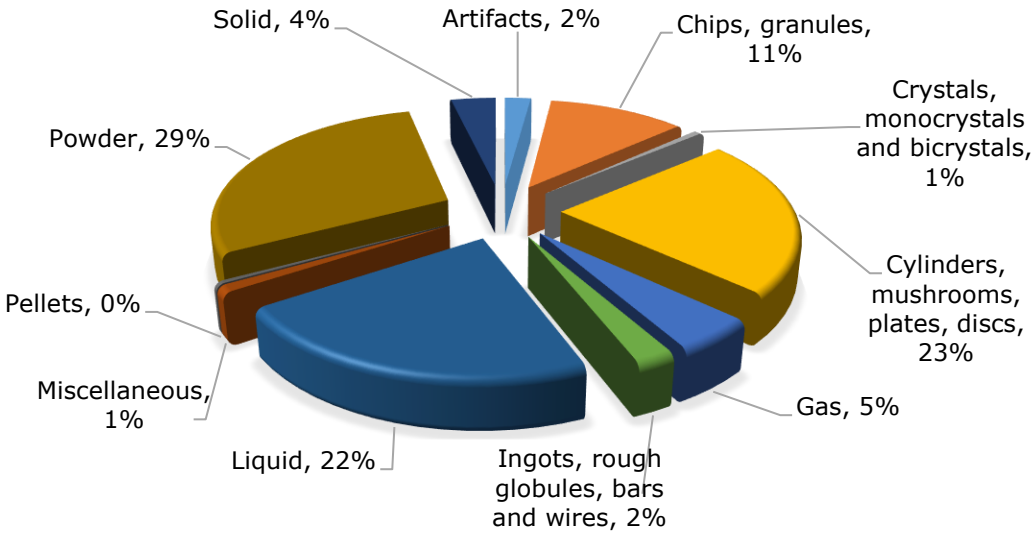

Figure 2: Origin of RMs at the level of COMAR database (27).

For each RM, the available information is the name, status (available or not), year, country, producer, general description, fields of application, and certified properties (27).

The selection of an RM by the user depends on his needs and objectives (2). The choice of an RM by the user takes into account the nature of the matrix, its concentration, availability, lifetime, and cost.

5.2. Constraints Related to The Production of Reference Materials

The production and certification of the RMs require enormous costs. These latter are linked to the initial investment for the purchase of the equipment, the time required for their development, the costs related to the certification procedure, as well as those about the production of a large stock needed to ensure the continuity of the availability of a specific lot over several years (23). Having accurate information on producer turnover and the cost price of the RMs is not easy. Nevertheless, the cost of R&D in the case of an RM is weakly echoed by the cost price in the case of the national metrology laboratories (1). This situation remains valid even for the big CRM producers such as NIST and IRMM (1).

RM users are confronted with the problem of identifying the appropriate material to use. Users are expected to look for an available RM, that also meets their needs (e.g., components, uncertainty, etc.) (28). In many cases, the available RMs are not suitable since they are not sufficiently similar to the real sample (3). The RMs that are available and adapted to the complexity and multiplicity of testing are insufficient (3). Also, new complex matrices need to be analyzed, and the producers are unable to rapidly produce CRMs for these new samples (3). As a result, CRM request exceeds supply in terms of availability and nature of materials. This encourages users and accreditation organizations to understand the limits of the RMs used (6).

5.3. Accreditation of Reference Materials Producers

The producers of RMs can be accredited by the standard ISO 17034 which specifies the general requirements for the competence of the producers of RMs. To get an overview of this standard in process form, we propose a process mapping bringing together all the elements covered by ISO 17034:2016 (10) (Figure 3).

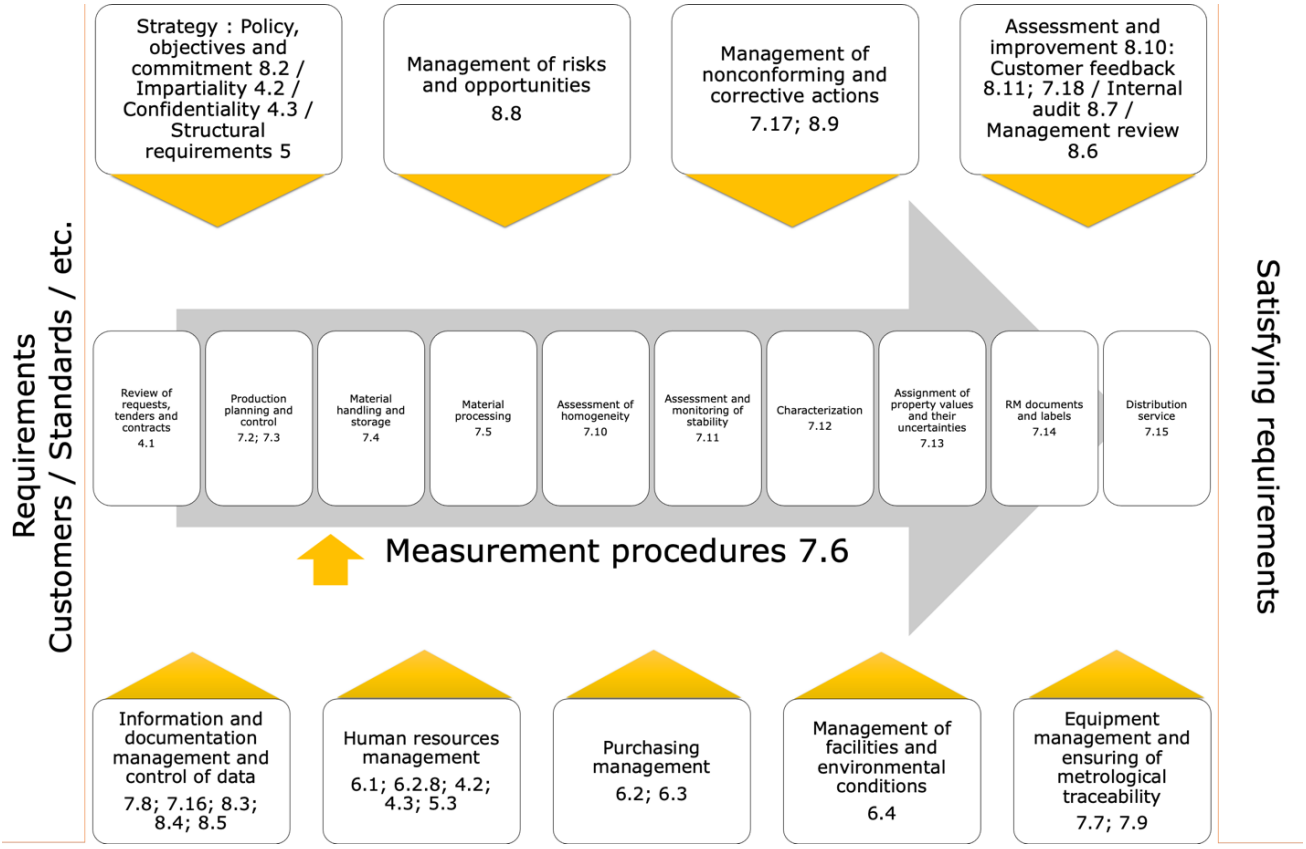

Figure 3: Process mapping bringing together all the elements covered by ISO 17034:2016.

ISO/IEC 17025 is considered indispensable for the application of the ISO 17034 standard which is aligned with the relevant requirements (10). Reference materials producers that comply with this international standard (ISO 17034) will also operate generally by the principles of ISO 9001 (10). The Cofrac, an accrediting organization, also takes into account the requirements of the standards ISO/IEC 17025 (25) and/or ISO 15189 (29) to accredit these producers (19). Table 1 presents common elements between ISO 17034:2016 (10), ISO/IEC 17025:2017 (25), and ISO 9001:2015 (30).

Table 1: Common elements between ISO 17034:2016, ISO/IEC 17025:2017, and ISO 9001:2015.

ISO 17034:2016	ISO/IEC 17025:2017	ISO 9001:2015
4.1 Contractual matters	7.1 Review of requests, tenders, and contract	-
4.2 Impartiality	4.1 Impartiality	-
4.3 Confidentiality	4.2 Confidentiality	-
5 Structural requirements	5 Structural requirements	-
6.1 Personnel	6.2 Personnel	-
6.2 Subcontracting 6.3 Provision of equipment, services, and supplies	6.6 Externally provided products and services	-
6.4 Facilities and environmental conditions	6.3 Facilities and environmental conditions	-
7.6 Measurement procedures	7.2 Selection, verification, and validation of methods 7.3 Sampling 7.7 Ensuring the validity of results	-
7.7 Measuring equipment	6.4 Equipment	-
7.8 Data integrity and evaluation	7.11 Control of data and information management	-
7.9 Metrological traceability of certified values	6.5 Metrological traceability	-
7.16 Control of quality and technical records	7.5 Technical records	-
7.17 Management of non-conforming work	7.10 non-conforming work	-
7.18 Complaints	7.9 Complaints	-
8.2 Quality policy	8.2 Management system documentation	5.2 Policy 6.2 Quality objectives and planning to achieve them
8.3 General management system documentation 8.4 Control of management system documents 8.5 Control of records	8.3 Control of management system documents 8.4 Control of records	7.5 Documented information
8.6 Management review	8.9 Management review	9.3 Management review
8.7 Internal audits	8.8 Internal audits	9.2 Internal audits
8.8 Actions to address risks and opportunities	8.5 Actions to address risks and opportunities	6.1 Actions to address risks and opportunities
8.9 Corrective actions	8.7 Corrective actions	10.2 non-conformity and corrective actions
8.10 Improvement	8.6 Improvement	10.3 Continual improvement
8.11 Feedback from customers	8.6 Improvement	9.1.2 Customer satisfaction

Among the mandatory requirements of ISO 17034:2016 (10) about the production of RMs, there are requirements for subcontractors. If the organization decides to call on the services of a subcontractor, the standard specifies the services that may or may not be subcontracted. These services are presented in Table 2.

Table 2: Services that can be subcontracted or not.

Activities that can be subcontracted	Processes should not be subcontracted
Sampling	Production planning
Processing an RM	Selection of subcontractors
Handling an RM	Assignment of proper values and their uncertainties
Homogeneity and stability testing	Authorization of property values and their uncertainties
Characterization	Authorization of RM documents
Storage of RM	
Distribution of RM	

When a reference material producer calls on a subcontractor to carry out part of the production, it must have procedures in place to ensure that the subcontractors' experience and technical competence are sufficient for the tasks entrusted to them and that they comply with the relevant sections of ISO 17034:2016 (10) and other appropriate standards.

6. DEVELOPMENT OF REFERENCE MATERIALS: KEY STEPS

6.1. Preparation of The Material

The RM preparation is performed in a manner that minimizes heterogeneity and instability (31). Ensuring RM stabilization is the most sensitive and difficult step (3). Many operations can be performed to avoid material changes (e.g., to avoid chemical or microbiological changes in the material, drying, sterilization by irradiation, and/or freezing can be used, among others). The nature of the material and the purpose of its use guide the choice of the method to be adopted (3). This choice must be made to respect, as far as possible, the integrity of the material (3). To avoid contamination, measures must be taken not to cause inhomogeneity in a material that is already homogeneous (31). Also, precautions must be taken to prevent any negative influence from the environment. After homogenizing the material, its storage must be done in suitable containers (3) (waterproof and inert (31)).

Homogeneity and stability are two primordial characteristics of an RM. The most important measures taken to ensure them are those taken during the selection, preparation, and packaging of the material (32). The testing realized at a later step over a limited period of some units of the RM can only verify the success or failure of previously performed steps (32).
The 2017 version of ISO Guide 35 (9) leaves to the RMs producers the judgment of the need or not to carry out experimental studies of homogeneity and stability based on several elements, namely (32):

- The knowledge of the physical, chemical, and/or biological properties of the material as well as the knowledge of the variation of these properties over time based on the scientific literature;
- The analysis of previous experimental data or monitoring data of stability;
- The absence of anomalies detected by the producer and the users further to cumulative observations on complete lifetimes;
- The tests were performed on the effect of the packaging.

6.2. Homogeneity Study

There are two types of homogeneity tests (5):

- Within-unit homogeneity, which represents the uniformity of a specified property value within each unit of an RM;
- Between-unit homogeneity represents the uniformity of a specified property value among units of an RM.

For the development of RMs, a within-unit and between-unit homogeneity study is carried out (4). The objective is to ensure that the content is the same in one unit and from one unit to another (3).

Different designs can be used for homogeneity studies, namely: simple randomized design, randomized block design, and nested design (9). Concerning the number of samples, it is recommended to take 10 to 30 samples from the batch of material, ISO Guide 35:2017 proposes a formula to calculate this number (9). Three repetitions per sample are advised; it is recommended to analyze them in a random order and not in the same order (4). The analyses must be carried out following the conditions of repeatability. The types of sampling to be performed can be systematic, random, or stratified random (4).

The study of homogeneity is carried out after placing the material in the containers (33). The authors evaluated the between-unit homogeneity by analyzing samples whose number varied between 6 and 16; this number was between 2 and 5 concerning the sub-samples used for the study of the within-unit homogeneity (34–43). The kind of sampling used is random (35–38,40,41,43) or stratified random (34,39). For the homogeneity study, internal (42,44) or external (33,34) standards can be used. The testing of samples is performed in a random order to distinguish between an analytical drift and a possible tendency in the filling sequence (37).

Homogeneity can be assessed using the ANOVA test (33,35–39,41,44–47). Other authors have used the Student's t-test to evaluate significant differences between averages obtained from within-unit and between-unit analysis (34,48). The outliers in the homogeneity study can be detected by visual inspection (9) or using the Grubbs test (9,37,38,44–46). Some authors used regression analysis to reject the drift of the measurement (36). The standard ISO 13528:2022 (49) recommends the use of the Cochran test for repetitions. Other standards provide several methods for identifying outliers, namely: ISO 5725-2:2019(50) (which describes Grubbs tests) and ISO 16269-4(51) (which describes graphical outlier tests and other tests for multiple outliers).

Concerning uncertainty associated with heterogeneity (w_{hom}), further to ISO Guide 35:2017 (9), it can be calculated by Formula:

$$u_{hom} = \sqrt{u_{bb}^2 + u_{wb}^2}$$

With,
u_{bb}: standard uncertainty associated with between-unit variability
u_{wb}: standard uncertainty associated with within-unit heterogeneity

If a basic design and one-way analysis of variance are used to evaluate homogeneity (figure 4), s_{bb}^2 (between-unit variance from a homogeneity study), is identical to the (squared) between-unit

homogeneity contribution to the uncertainty, u_{bb}^2 (9). In the case presented in Figure 4, the s_{bb}^2 we will obtain from ANOVA 1 in the case of within-unit homogeneity will be equivalent to s_{wb}^2 (the second component of the uncertainty u_{hom} (u_{wb}^2)).

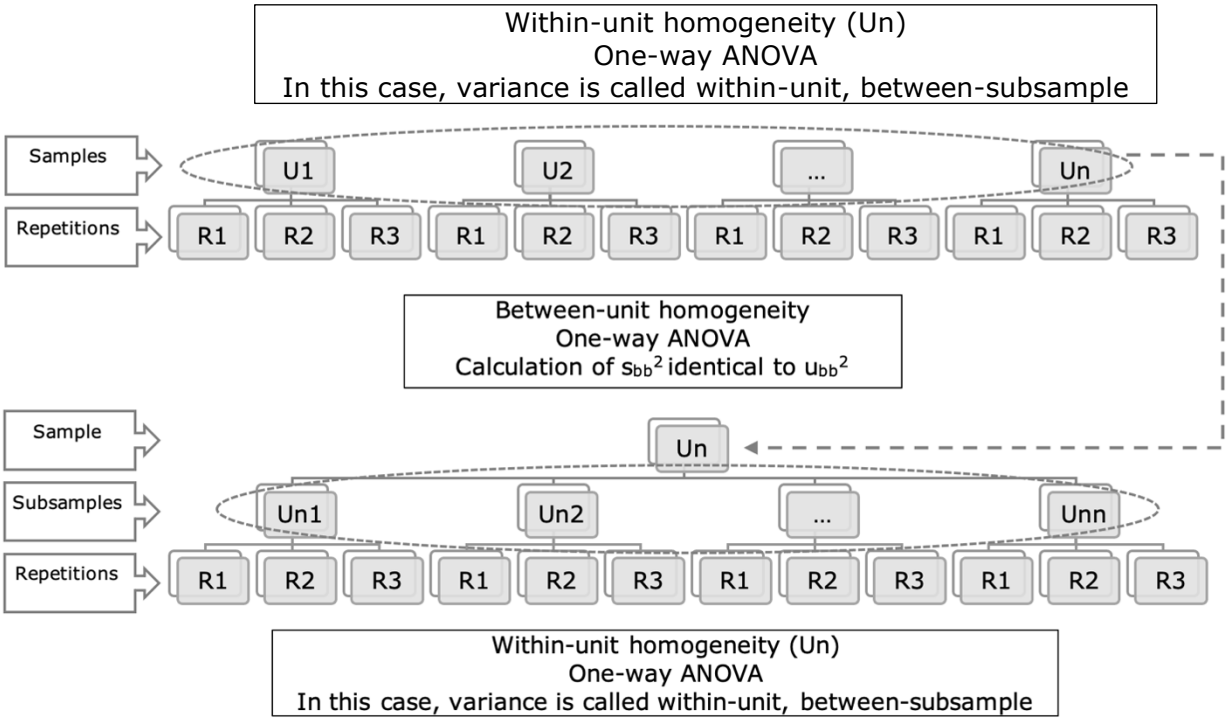

Figure 4: Diagram of a basic plan for carrying out a between-unit and within-unit homogeneity study.

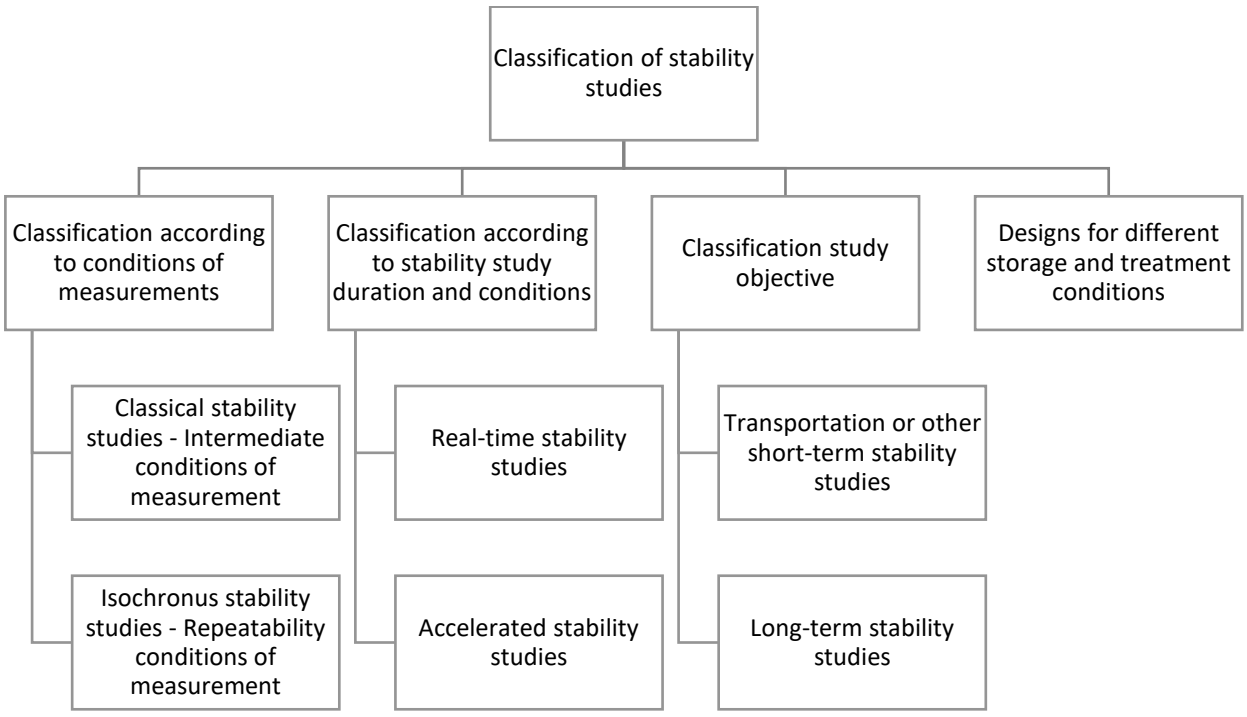

Figure 5: Classification of stability studies according to ISO Guide 35:2017.

Some authors (37) have used the method described by Van Der Veen, Linsinger, & Pauwels (52) to calculate the uncertainty due to possible inhomogeneity which can be masked by the repeatability of the method.

6.3. Stability Study

The properties of the material and the parameters studied should remain unchanged over long periods (3). For that purpose, a stability study is performed to evaluate the behavior of the material under various conditions. In general, a series of measurements realized at different times is performed to accomplish a stability study (31). Figure 5 presents the classification of stability studies according to ISO Guide 35:2107 (9).

ISO 17034:2016 (10) requires stability to be assessed under the proposed conditions of transport and the proposed conditions of storage. This assessment can be carried out either accelerated or in real-time, using a classical or isochronous study. The latter is defined by ISO Guide 35:2017 (9) as an "experimental study of reference material stability in which units exposed to different storage conditions and times are measured in a short period".

A short-term stability study is generally performed to determine conditions during delivery (3,37,41,44,46). Habitually high temperatures (between 35 and 40 °C) are used to assess predictable degradation under the most unfavorable conditions, such as during transport (3). The long-term stability helps to identify optimal storage conditions for the RM (37,41,44). An accelerated stability study (where some samples are exposed to extreme conditions in comparison to desired storage or transport conditions) has the advantage of reducing the total time required for the study of experimental stability (32). Some authors suppose that a "sample stable at + 40 °C during one year may be stable at + 20 °C for a longer period (Arrhenius)" (3). Other authors judge the simulation of long-term storage under difficult conditions as inappropriate, given the possibility of modification of the degradation mechanism (31). Therefore, using extrapolation to estimate stability data from higher temperatures using the Arrhenius equation is not recommended (31).

To study the stability of the RM, the authors analyzed samples of the RM stored at expected temperatures for storage, extreme temperatures (e.g., 40 °C, 60 °C), and temperatures where it is supposed to have very weak or negligible variations of the RM (e.g., -20 °C, -70° C, and -80° C) (3,31,33–35,37,46). The stability study can also be performed by modifying other conditions, such as light, or darkness (53) and/or humidity. The short-term stability was assessed by the authors over periods ranging from 0, 1, and 2 weeks, then over regular intervals of one month to 4 months (33–35,37,38). The study of long-term stability has been carried out between 3 months and 2 years for some authors (33–35,37,38,46,54,55). The number of samples analyzed at each time/temperature pair varied between 1 (56), 2 (33,57), 3 (46), 5 (35) and 6 (37) samples. Certain authors use an external calibration for the study of stability (33). The evaluation of stability is carried out by comparing the obtained results in the initial time with those obtained in each period (35). Some authors (34,37,57,58) used the isochronous approach (59) to assess the stability of the RM.

The stability study is performed by analyzing samples chosen randomly (33,46). Increasing the number of repetitions per time and increasing the duration of the stability study allow us to reduce the influence of the analytical variation (31). To assess stability, certain authors have used the F test (54). The Grubbs test is used by other authors to identify outliers (37,44). The latter also performed linear regression analysis as a function of time and for each temperature. These same authors tested the slopes for their significance using a t-test proposed also by ISO Guide 35:2017 (9). The uncertainty of the stability has been estimated by some authors (37,44) "as the uncertainty of the regression line with a slope of zero multiplied by the envisaged shelf-life of the material", as described by Thomas P.J. Linsinger et al. (60).

6.4. Characterization and Evaluation of Measurement Uncertainty

According to ISO Guide 30:2015 (5), the characterization of an RM is the "determination of the property values or attributes of a reference material, as part of the production process". It aims to obtain a metrologically valid estimation of the real value of the property (61).

There are several valid characterization approaches (61):

- A single (primary) method in a single laboratory;
- Two or more independent reference methods in one or several laboratories;
- One or more methods of demonstrable accuracy, performed by a network of competent laboratories;
- An approach providing method-specific, operationally defined property values, using a network of competent laboratories.

The Consultative Committee for Amount of Substance (CCQM) defined the primary method of measurement as a "method having the highest metrological qualities, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units, and whose results are, therefore, accepted without reference to a standard of the quantity being measured" (62). The VIM (63) defined the primary reference measurement procedure as a "reference measurement procedure used to obtain a measurement result without relation to a measurement standard for a quantity of the same kind".

Some authors consider that the primary method is completely understood, so they judge that a single value from a single laboratory is sufficient since an unknown laboratory or method bias can be excluded (61). Other authors say that the laboratory may have a bias, and therefore, by using only one method in a single laboratory, the certified value may be wrong (3). In addition, an estimation of the uncertainty may not be correct by choosing the use of the results of one method in a single laboratory (3).

On the other hand, the reference method is defined in ISO Guide 30:2015 (5) as a "measurement method, that has been shown to have the appropriate trueness and precision for its intended use and has been officially defined as reference method by a competent body". The VIM (63) defines the reference measurement procedure as a "measurement procedure accepted as providing measurement results fit for their intended use in assessing the measurement trueness of measured quantity values obtained from other measurement procedures for quantities of the same kind, in calibration, or in characterizing reference materials".

The use of several independent methods is the most commonly adopted approach (3,21).

The choice of the characterization of the samples by several laboratories is within the scope of the interlaboratory comparisons. This involves sending the same entity or similar entities to the selected laboratories for analysis. Further to the obtained results by the different laboratories, a statistical analysis is planned to identify the assigned values and their associated uncertainties. ISO 13528 presents the statistical methods that can be used in proficiency testing by interlaboratory comparison. Interlaboratory comparisons allow us to verify the trueness of the methods of analysis (2).

The number of chosen laboratories (6, 11,24), the number of used methods (1, 2, 3), the number of analyzed samples (2), the number of repetitions per sample (1, 2, 6) as well as the period of analysis (2 days, 3 days) differ from one author to another (4,41,44). Some authors chose to do the repetitions under intermediate precision conditions (4). For the control of the measurements, the authors have chosen to send also two samples of a "standard certified calibration" for analysis (41). The selected laboratories must use validated methods and be able to ensure the traceability of results (4).

Evaluation of the uncertainty is carried out following the characterization. This assessment takes into account the uncertainty of the testing having allowed the determining of reference values (characterization) as well as those related to within-unit and between-unit homogeneity and stability (2,39). These last two uncertainties may not appear in the uncertainties budget if the contribution of the one related to homogeneity is shown to be negligible and if the stability of the property value of the material can be insured (64).

To help RM producers in preparing the documentation that will accompany them, the ISO has established ISO Guide 31 (7) which lists the information that must be included on a certificate of a CRM, on the label attached to the container, and also on the product information sheets. This information will help the users to confirm the adequacy of the chosen CRM.

7. CONCLUSION

The importance of the reference materials has pushed among others the ISO to the establishment of several standards concerning the development and use of the reference materials.

The development of the reference materials consists of four steps mainly: preparation, realization of the homogeneity study, carrying out the stability study, and characterization. These steps are equally important; the fact of highlighting less than one of them will imply a significant decrease in the quality of the reference material developed.

The production of reference materials is governed by many different factors and may differ from one country to another. The introduction of quality assurance in laboratories, the implementation of a quality approach by ISO/IEC 17025, the regulatory framework which sometimes imposes thresholds for certain elements as well as the demand of industrialists can be considered elements encouraging the production of the reference materials. These latter are of paramount importance in analysis laboratories since they have several roles, namely calibration of equipment, development, and validation of methods, and realization of interlaboratory comparisons among others. Certainly, the laboratories need more and more reference materials, however finding the appropriate ones is not easy. In several cases, the available reference materials do not correspond 100 % to the sample to be analyzed because of the variety of compounds, concentrations, and matrices; this makes the development of new reference materials a necessity. This expensive operation requiring technical knowledge and important experience makes the price of these reference materials high, which makes their use limited in the laboratories.

The outlook for the reference materials development market will depend on several factors, including the rapid development of analytical technologies using cutting-edge analytical methods, and the expansion of the biotechnology, nanotechnology, and renewable energy sectors. Demand for sustainably produced reference materials could increase as environmental concerns gain in importance. Increasingly stringent standards and regulations, economic change, and scientific developments could lead to a customization of demand for reference materials tailored to the specific needs of certain fields.

8. CONFLICT OF INTEREST

Nothing to report.

9. ACKNOWLEDGMENTS

Nothing to report.

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