



Guidelines for the Selection and Use of Reference Materials

ILAC-G9:2005

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To obtain permission or for further assistance, please contact:

The ILAC Secretariat

c/- NATA

PO Box 7507

Silverwater NSW 2128

Australia

Fax: +61 2 9736 8373

Email: ilac@nata.asn.au

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PREAMBLE

This document replaces ILAC G9:1996 Guidelines for the Selection and Use of Certified Reference materials. It has been revised and expanded to address the increasing volume of information which is now available on this topic.

PURPOSE

The aim of this paper is to provide a short, simple and user-friendly guide for laboratories, and accreditation and certification bodies. It employs mainly VIM (1) and ISO (2) definitions and provides references to other more comprehensive and expert texts. It is intended to provide help for the inexperienced rather than the expert and necessarily simplifies some topics. It includes discussion and clarification of some poorly understood issues. Although based on the requirements of chemical measurement, it is intended to be also of use in other areas of measurement.

AUTHORSHIP

This document was prepared by the ILAC Accreditation Committee in association with the ILAC Laboratory Committee, and endorsed by the ILAC Membership.

INTRODUCTION

There are a number of authoritative and detailed documents on various aspects of reference materials and these are listed at the end of this paper, together with some internationally recognised (1-4) definitions. This paper aims to provide a simple guide on the use of reference materials (RMs) within a wider quality programme. Reference materials are an important tool in realising a number of aspects of measurement quality and are used for method validation, calibration, estimation of measurement uncertainty, training, internal quality control (QC) and external quality assurance (QA) (proficiency testing) purposes.

In the wider sense, the validity of measurements can be assured when:

- ◆ validated methods and appropriate equipment are used
- ◆ qualified and competent staff undertake the work
- ◆ comparability with measurements made in other laboratories is assured (traceability and measurement uncertainty)
- ◆ independent evidence of performance is available (proficiency testing)
- ◆ well defined QC and QA procedures are employed, preferably involving third party accreditation

Often a measurement operation serves more than one quality purpose and there can be overlap of function as illustrated in Figure 1 (Page 14). Different types of reference materials are required for different functions. For example, a certified reference material would be desirable for method validation but a working level reference material would be adequate for QC.

More detailed guidance on the QA of chemical measurements, including cover of reference materials, calibration, QC and validation is provided in a joint CITAC Eurachem guide (5).

TYPES OF REFERENCE MATERIALS

RMs are used to support measurements concerned with chemical composition, biological, clinical, physical, engineering properties and miscellaneous areas such as taste and odor. They may be characterised for 'identity' (e.g. chemical structure, fiber type, microbiological species etc.) or for 'property values' (e.g. amount of specified chemical entity, hardness etc.). Some commonly encountered types of reference materials include:

1. **Pure substances** characterised for chemical purity and/or trace impurities.
2. **Standard solutions** and gas mixtures, often prepared gravimetrically from pure substances and used for calibration purposes.
3. **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.
4. **Physico-chemical reference materials** characterised for properties such as melting point, viscosity, and optical density.
5. **Reference objects or artefacts** characterised for functional properties such as taste, odour, octane number, fl ash point and hardness. This type also includes microscopy specimens characterised for properties ranging from fibre type to microbiological specimens.

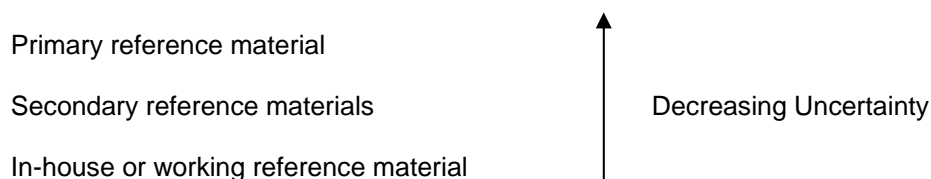
CLASSIFICATION OF REFERENCE MATERIALS

Two classes of materials are recognised by ISO, namely 'certified reference materials' (CRMs) and 'reference materials' (RMs). CRMs must by definition be traceable to an accurate realisation of the unit in which the property values are expressed. Each property value must be accompanied by an uncertainty at a stated level of confidence. RMs are materials whose property values are sufficiently homogeneous and well established to be used for the 29/03/2005 calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

For most chemical reference materials produced before the late 1990s, the measurement uncertainty values given by the producers are unlikely to have been estimated by the now recommended ISO (6,7) procedure. The actual uncertainty can be expected to be larger than stated by a factor of 2-3, where only within laboratory precision measurements are used and by a smaller factor where certification included a range of validated methods and a number of laboratories.

Some materials sold as CRMs have no stated evidence of traceability, and in such cases the user should make a judgement on the possible traceability of the value assigned to the CRM.

The following classes of reference materials may be encountered:



Other terminology, such as NIST Standard Reference Materials (SRMs) is also used and a classification (class O - V) based on degree of traceability to SI has been proposed (8). Pan (9) has also published a useful paper on this topic.

TRACEABILITY OF REFERENCE MATERIALS

A short review of the concept and practice of traceability in chemical measurement is contained in the EURACHEM/CITAC guide on quality (5). An in-dept treatment of the topic is given in the CITAC/ EURACHEM Guide on traceability of measurement results (5A). Reference materials are important tools for the transfer of measurement accuracy between laboratories and their property values should, where feasible, be traceable to SI. Traceability is, however, a relatively new concept in the field of chemical measurement and as a consequence very few chemical reference materials are explicitly traceable to SI. A hierarchy of methods is, however, used for assigning property values to materials and even if not stated, their traceability can be described as follows:

Measurement Method	Traceability
Primary method	SI
Method of known bias	SI/International standard
Independent method(s)	Results of specified methods
Inter-laboratory comparison	Results of specified methods

A combination of value assignment procedures is sometimes employed, such as a consensus value derived from an inter-laboratory comparison where primary methods were used. In the absence of formally stated traceability it will be necessary for the user to make judgements about implicit traceability, based on the data available in reports and the technical literature. It is important to ensure that chemical interferences and matrix effects are adequately addressed in arriving at both the certified value and its uncertainty. Unknown levels of bias are not uncommon and contribute to lack of agreement of measurements.

The measurement uncertainty of the property value of a reference material employed in a measurement process, will contribute to the uncertainty of the final measurement but should contribute less than one third of the overall measurement uncertainty. Any underestimation of the uncertainty of the RM property value will, of course, be carried through to measurements where the RM is used.

THE AVAILABILITY AND SELECTION OF REFERENCE MATERIALS

Generally the demand for reference materials exceeds supply in terms of the range of materials and availability. It is rare to have a choice of alternative RMs and the user must choose the most suitable material available. It is important therefore that users and accreditation bodies understand any limitations of reference materials employed.

There are, however, several hundred organisations producing tens of thousands of reference materials worldwide. Producers include internationally renowned institutions such as NIST; collaborative government sponsored programs such as the EU BCR program, semi-commercial sectoral or trade associations such as the American Oil Chemicals Association and an increasing number of commercial organisations. The distinction between government institutes and commercial businesses is disappearing with the privatisation of a number of national laboratories.

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. In the absence of data being provided by the supplier that would underpin the assigned property value, it is the responsibility of the user to assess the information available and undertake further characterisation as appropriate. Guidance on the preparation of reference materials is given in ISO Guides 31, 34 and 35 and guides on the preparation of working level reference materials are also available (12,13).

Information about reference materials is available from a number of sources. Some examples include: The COMAR Database contains information on more than 10,000 RMs/CRMs. Further information can be obtained from the COMAR Central Secretariat (www.comar.bam.de).

A database has been prepared by CITAC and ISO REMCO (14) and it covers reference materials currently under development.

The BIPM have developed two data bases, one is categorised under Appendix C of the CIPM-MRA and the second under the remit of the Joint Committee for Traceability in Laboratory Medicine (JCTLM). Both data bases (www.bipm.org) will provide useful information on traceability of the assigned property values.

An internet database for a selection of RMs has been developed by the International Atomic energy Agency (IAEA), (<http://www.iaea.org/programmes/nahunet/e4/nmrm/index.htm>).

Virtual Institute on Reference Materials (VIRM) is an EU funded project which is intended to become self financing. It is web based and provides information on many aspects of RMs www.virm.org

A number of suppliers provide a comprehensive range of materials including materials produced by other organisations and aim to provide a one-stop-shop for users.

USES OF REFERENCE MATERIALS

There are many types of reference materials and some of the commonly encountered types are listed in the section “Types of Reference Materials”. Some examples of their uses are described in this section, but for a more detailed description of their various uses the reader should consult ISO Guides 32 and 33.

Method Validation and Measurement Uncertainty

Estimation of bias (the difference between the measured value and the true value) is one of the most difficult elements of method validation, but appropriate RMs can provide valuable information, within the limits of the uncertainty of the RMs certified value(s) and the uncertainty of the method being validated. Although traceable certified values are highly desirable, the estimation of bias differences between two or more methods can be established by use of less rigorously certified RMs. Clearly the RMs must be within the scope of the method in terms of matrix type, analyte concentration etc. and ideally a number of RMs covering the full range of the method should be tested. Where minor modifications to a well-established method are being evaluated then less rigorous bias studies can be employed.

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 (15). The uncertainty associated with an RM should be no greater than one third of that of the sample measurement.

Verification of the Correct Use of a Method

The successful application of a valid method depends on its correct use, both with regard to operator skill and suitability of equipment, reagents and standards. RMs can be used for training, for checking infrequently used methods and for trouble shooting when unexpected results are obtained.

Calibration

Normally a pure substance RM is used for calibration of the measurement stage of a method. Other components of the test method, such as sample digestion, separation and derivatisation are, of course, not covered and loss of analyte, contamination, interferences and their associated uncertainties must be addressed as part of the validation of the method. The uncertainty associated with RM purity will contribute 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.

Some other methods, such as XRF analysis, use matrix RMs for calibration of the complete analytical process. In addition to a close matrix match, 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 (12).

ISO Guide 32 and reference 7 provide additional useful information.

Quality Control and Quality Assurance (QC&QA)

RMs should be characterised with respect to homogeneity, stability, and the certified property value(s). For inhouse QC, however, the latter requirement can be relaxed, but adequate homogeneity and stability are essential. Similar requirements apply to samples used to establish how well or badly measurements made in different laboratories agree. In the case of proficiency testing, homogeneity is essential and sample stability within the time-scale of the exercise must be assessed and controlled. Although desirable, the cost of assigning the property values of proficiency testing samples often prohibits this being done and consensus mean values are often used instead. As a consequence, there often remains some doubt concerning the reliability of assigned values used in proficiency testing schemes. This is because, although the consensus mean of a set of data has value, 'the majority' may not necessarily be correct and as a consequence the values carry some undisclosed element of uncertainty. The interpretation of proficiency testing data thus needs to be carried out with caution.

Assessment of the Suitability of Reference Materials

As previously indicated the key parameter associated with the assigned value is the uncertainty and the reliability of the uncertainty estimate. Uncertainty budgets should be derived using the ISO approach (6,7). The assigned value should be stated together with the expanded uncertainty, U , using a coverage factor $k=2$. This provides a level of confidence of approximately 95%. However, the full uncertainty data is often not available and it is necessary to consider other criteria such as a quality check list, in order to ascertain the suitability of the RM for the task in hand. Users must, therefore, be cautious and seek clear evidence of the quality and traceability of the property values of materials from suppliers as detailed below.

In cases where all of the data pertaining to the assigned value and its associated uncertainty are available to the user, the non expert may not be in a position to evaluate it. Users must, therefore be cautious and seek clear evidence from suppliers of the traceability of the assigned property values of materials and the competence of the laboratories involved in that value assignment process. Ideally the RM should be produced in accordance with a recognised quality system references xyz and which

has been audited and received third party recognition Systems, for example based on ISO Guide 34, which contains normative reference to ISO/IEC 17025 (important for the assignment of the property value segment) are now in operation in many parts of the world, but it will take many years for these systems to impact significantly on the market place.

A protocol for assessing the suitability of RMs is detailed in Figure 2 (Page 15) and discussed below. The user must assess the appropriateness and fitness for purpose of any RM based on the customer and analytical requirements. Factors to be considered include the following:

1. The suitability of a reference material depends on the details of the analytical specification. Matrix effects and other factors such as concentration range can be more important than the uncertainty of the assigned value as detailed in (11). The factors to consider include:
 - Measurand including analyte
 - Measurement range (concentration)
 - Matrix match and potential interferences
 - Sample size
 - Homogeneity and stability
 - Measurement uncertainty
 - Value assignment procedures (measurement and statistical)
2. The validity of the data, relating to the assigned value and its uncertainty including conformance of key procedures with ISO Guide 35 and other ISO requirements (6,7).
3. Track record of both the producer and the material. For example, consideration of whether a RM in use has been subjected to an interlaboratory comparison, cross-checked by use of different methods, or there is experience of use in a number of laboratories over a period of years.
4. Availability of a certificate and report conforming to ISO Guide 31.
5. Demonstrated conformance of the production of the reference materials with quality standards such as ISO Guide 34 or ILAC requirements (10), or compliance of the measurement capability of property values with ISO/IEC 17025 (16) requirements.

All or some of the requirements may be specified in the customer and analytical specification, but often it will be necessary for analysts to use their professional judgment. Finally, quality does not necessarily equate to small uncertainty and fitness for purpose criteria need to be used.

Certificates and Supporting Reports

Ideally, a certificate complying with ISO Guide 31 and a report covering the characterisation, certification and statistical analysis procedures, complying with ISO Guide 35, will be available. However, many RMs, particularly older materials and materials not specifically produced as RMs, may not fully comply with ISO Guides 31 and 35. Alternative, equivalent information in whatever form it is available, that provides credible evidence of compliance can be considered acceptable. Examples include the following: technical reports, trade specifications, papers in journals or reports of scientific meetings and correspondence with suppliers.

Assessment of the Suitability of Reference Materials

Laboratories must be able to explain and justify the basis of selection of all RMs and of course any decision not to use a RM. In the absence of specific information it is not possible to assess the quality of an RM. The rigour with which an assessment needs to be conducted depends on the criticality of the measurement, the level of the technical requirement and the expected influence of the particular RM on the validity of the measurement.

Only where the choice of RM can be expected to significantly affect measurement results is a formal suitability assessment required.

IN-HOUSE PREPARATION OF REFERENCE MATERIALS

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 guides available (12,13) to help the non-specialist laboratory prepare their own RMs. Some of the key issues that need to be considered are: selection of materials (appropriateness, native material versus spikes, material preparation etc.), homogeneity testing, preparation and packaging (homogeneity, contamination, stability etc.), stability testing, value assignment exercises, uncertainty estimation, documentation and QA, mechanism for the approval of the assigned value, storage, and distribution.

DEFINITIONS

Calibration (1): Set of operations that establish, under specified conditions, the relationship between the values of quantities indicated by a measurement instrument or measuring system or values represented by a material measure or a reference material, and the corresponding values realised by standards.

Certified Reference Material (1): Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realisation of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

Primary method (2): A primary method is a method having the highest metrological qualities, whose operation can be completely described, and understood and for which a complete uncertainty statement can be written down in terms of SI units. A primary direct method measures the value of an unknown without reference to a standard of the same quantity. A primary ratio method measures the ratio of an unknown to a standard of the same quantity; its operation must be completely described by a measurement equation. The methods identified as having the potential to be primary methods are: isotope dilution mass spectrometry; gravimetry, covering gravimetric mixtures and “gravimetric analysis”; titrimetry; coulometry; determination of freezing point depression; differential scanning calorimetry and nuclear magnetic resonance spectroscopy. Other methods such as chromatography, which has extensive applications in organic chemical analysis, have also been proposed.

Reference material (RM) (1): Material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

Traceability (1): Property of a result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.

Uncertainty of measurement (1): Parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand.

Validation (3): Confirmation by examination and provision of objective evidence that the particular requirements of a specified intended use are fulfilled. Method validation has not been formally defined but a Guide on the topic is available (4).

KEY REFERENCE MATERIALS PUBLICATIONS

The following guides have been prepared by ISO REMCO, the international committee that leads on reference material matters:

- ISO Guide 30:1992 Terms and definitions used in connection with reference materials
- ISO Guide 31:2000 Contents of certificates of reference materials
- ISO Guide 32:1997 Calibration of chemical analysis and use of certified reference materials
- ISO Guide 33:2000 Uses of certified reference materials
- ISO Guide 34:2000 General requirements for the competence of reference material producers as amended by Technical Corrigendum 1 of 15/11/04
- ISO Guide 35:1989 (Under Revision) Certification of reference materials-General and statistical principles
- ISO/REMCO Document N 330 List of producers of certified reference materials, Information by Task Group 3 “Promotion”

Other guides include:

- European Commission Document, BCR/48/93 (Dec 1994): Guidelines for the production and certification of BCR reference materials
- NIST Publication 260-100 (1993): Standard Reference Materials - Handbook for SRM Users
- IUPAC ‘Orange Book’: Recommended Reference Materials for the Realisation of Physico chemical Properties, Edited K N Marsh, Blackwell Scientific Publications, 1987
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FIGURE 1: OVERLAP BETWEEN FUNCTIONS ASSOCIATED WITH MEASUREMENT TRACEABILITY AND ANALYTICAL QUALITY

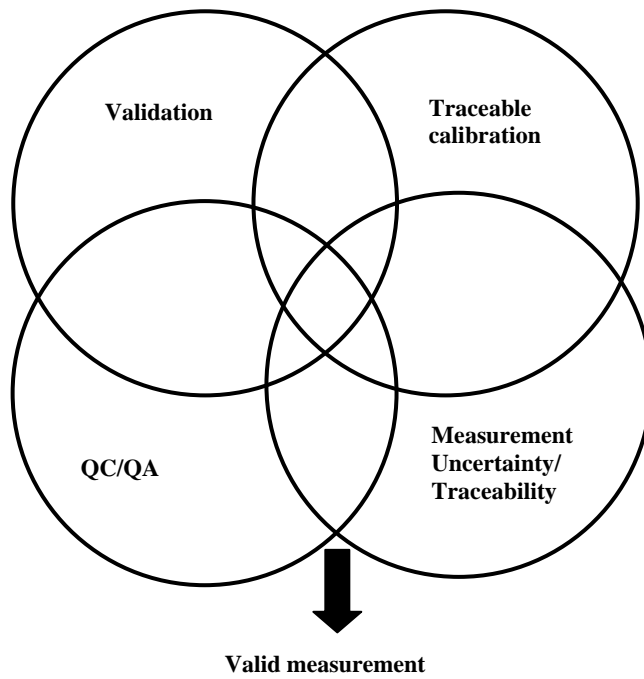
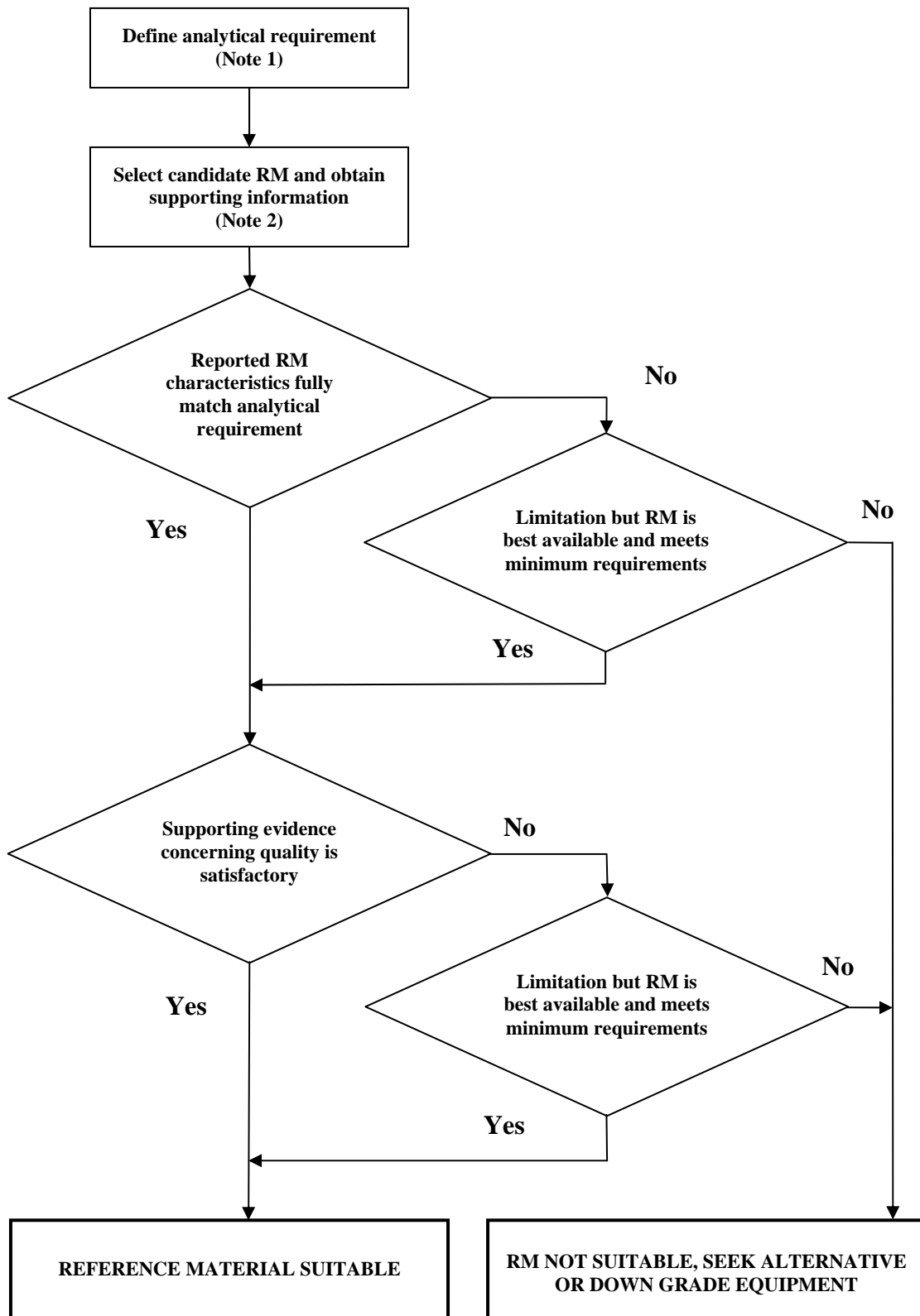


FIGURE 2: ASSESSMENT OF THE SUITABILITY OF A REFERENCE MATERIAL



Notes to Fig. 2:

Note 1: The analytical requirements specification should include details concerning the measurand, concentration, traceability, measurement uncertainty, etc

Note 2: Key characteristics should be available in the RM Certificate. Additional information, for example details of the method(s) used for value assignment and the full measurement uncertainty budget should also be available in the Certificate or in a supporting report.