THE SELECTION AND USE OF REFERENCE MATERIALS

A BASIC GUIDE FOR LABORATORIES AND ACCREDITATION BODIES

ABSTRACT

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 ISO (3) and VIM (1) 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 misunderstood issues. Although based on the requirements of chemical measurement, it is intended to be also of use in other areas of measurement.

INTRODUCTION

There are a number of authoritative and detailed texts 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 and for internal quality control (QC) and external quality assurance (QA) (proficiency testing) purposes.

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

- work is carried out to a clearly defined customer requirement
- validated methods and 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. 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 odour. They may be characterised for 'identity' (e.g. chemical structure, fibre type, microbiological species etc.) or for 'property values' (e.g. amount of specified chemical entity, hardness etc.). Some commonly encountered types of reference materials are as follows:

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, flash 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 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. Also some materials sold as CRMs have no stated traceability.

The following classes of reference materials are also encountered:

Primary reference material

Secondary reference materials   Decreasing Uncertainty

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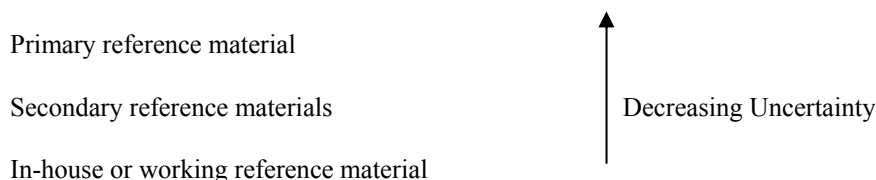
Other terminology, such as NIST Standard Reference Materials (SRMs) is also used and a classification (class O - V) based on degree of traceability to SI has been proposed (8). Pan (9) has also published a useful paper on this topic.

An internationally recognised quality approval system to assure the competence of RM producers (based on ISO Guide 34 and reference 10) is under consideration within the International Laboratory Accreditation Co operation, but it will take some years for any system to make a significant impact on the market place. 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.

TRACEABILITY OF REFERENCE MATERIALS

A short review of the concept and practice of traceability in chemical measurement is available in reference 5. 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
Interlaboratory comparison	Results of specified methods

A combination of certification procedures is sometimes employed, such as a consensus value derived from an interlaboratory 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 certification 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 certification data provided by the supplier 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. The COMAR Database, contains information on more than 10,000 RMs/CRMs which can be accessed directly or through institutes which provide an advisory service. Further information can be obtained from the COMAR Central Secretariat (comar@BAM.de). Advisory services assist users identify the type of material required for their task and identify a supplier. A database covering reference materials currently under development has been prepared by CITAC and ISO REMCO (14). 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. A first internet database for a selection of RMs has recently appeared and is likely to be followed by others (http://www.iaea.org/programmes/nahunet/e4/nmrm/index.htm) provided by some RM providers.

USES OF REFERENCE MATERIALS

A more detailed description of the use of RMs is available in 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 and 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 in-house 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 certifying 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' is not necessarily 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 quality parameter is the uncertainty associated with the certified value and the reliability of the uncertainty estimate. Uncertainty budgets should be derived using the ISO approach (6,7). Certification data should be stated together with the expanded uncertainty, U, using a coverage factor k=2 which gives a level of confidence of approximately 95%.

However, the full uncertainty data is often not available and it is necessary to consider other quality criteria. Also, the non-expert may not be in a position to fully evaluate the certification data and a quality check list, or a third party quality approval system, is desirable. Such systems are under development but may take some time to become fully established.

A protocol for assessing the suitability of RMs is detailed in Figure 2 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 certified 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 certification and uncertainty data, 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, when 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 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 the analyst to use professional judgement. 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, certification studies, uncertainty estimation, documentation and QA, certification approval, storage, distribution and relifing.

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

- ISO Guide 35:1989 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

- World Health Organisation (WHO) Guidelines for the Preparation and Characterisation and Establishment of International and other Standards and Reference Reagents for Biological Substances, Technical Report Series No 800 (1990)

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2. Minutes of 4th CCQM meeting, Feb 1998, Paris

3. ISO 9000, Quality management systems - Fundamentals and vocabulary (ISO 9000:2000);

4. Eurachem Guide: The fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 1998, Published by LGC UK (see www.eurachem.org)

5. CITAC Eurachem Guide , Guide to Quality in Analytical Chemistry, An Aid to Accreditation, 2001, In press – see Eurachem or CITAC web site.

6. Guide to the expression of uncertainty in measurement, first edition, 1995, ISO/BIPM/IEC/IFCC/IUPAC/IUPAP/IOML. (Published by ISO)

7. Eurachem/CITAC Guide, Quantifying Uncertainty in Analytical Measurement, 2. edition 2000, Published by LGC, UK (see www.eurachem.org)

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11. A Marschal, Traceability and Calibration in Analytical Chemistry - Principles and Applications to Real Life in Connection with ISO 9000, EN 45000 And ISO Guide 25, Eurolab Symposium, Firenze, April 1994

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13. J M Christensen, Guidelines for Preparation and Certification of Reference Materials for Chemical Analysis in Occupational Health, NORDREF, 1998 (ISBN: 87-7904-010-1)

14. S D Rasberry and C L Monti, Worldwide Production of CRMs: 1996 Status Report, NIST Report Feb 1996

15. S L R Ellison and A Williams, Measurement Uncertainty: The Key to the Use of Recovery Factors, pp 30-37, The Use of Recovery Factors in Trace Analysis, Ed M Parkany, RSC, 1996

16. ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:1999)

Prepared by Bernard King on behalf of and with assistance from the EEE-RM working group.

Figure 1: Overlap between functions associated with Measurement Traceability and Analytical Quality
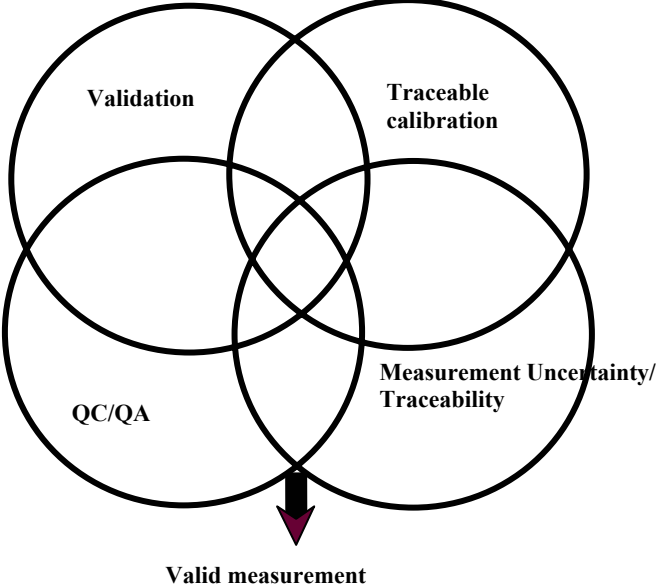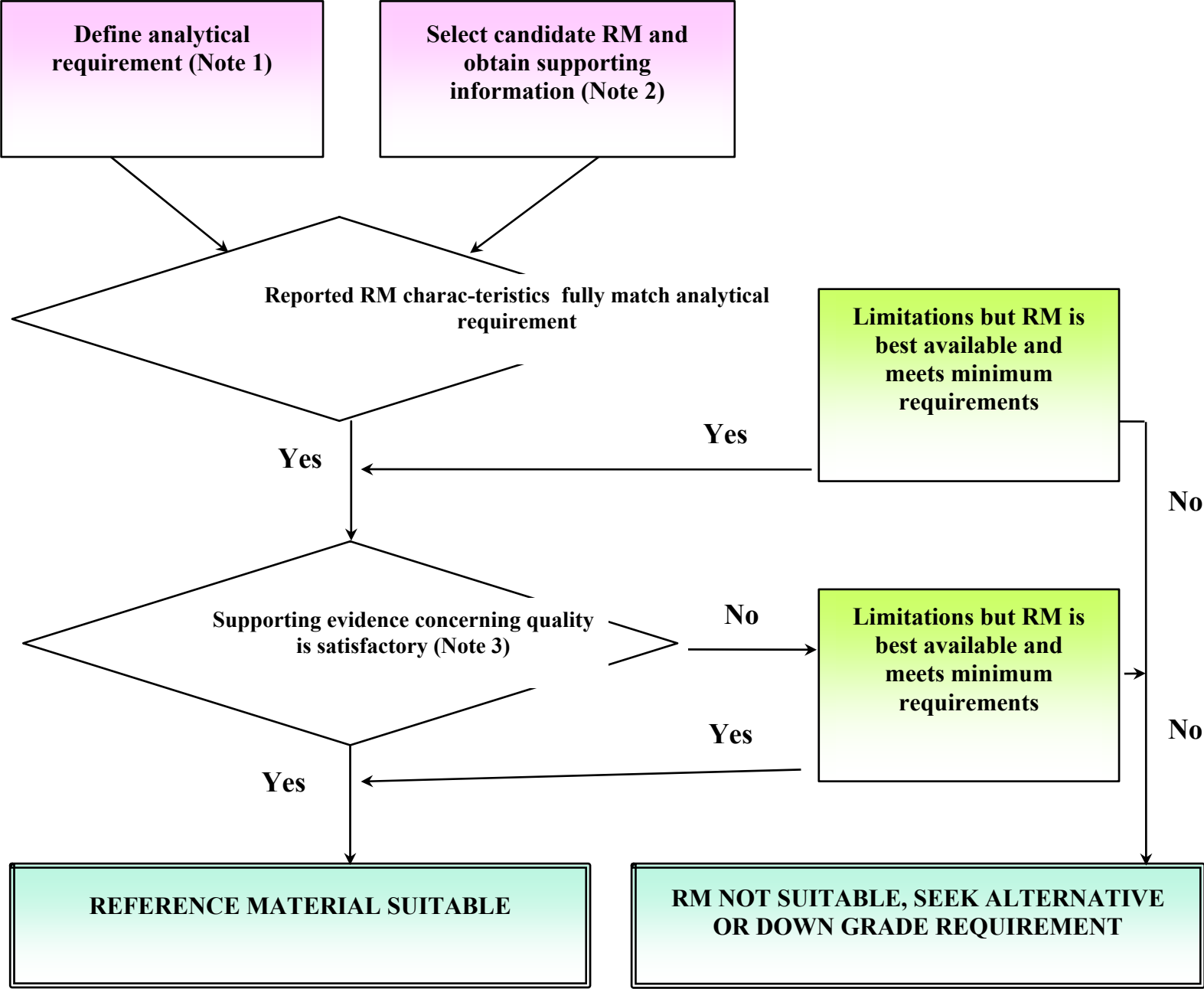

Figure 2: Assessment of the suitability of a Reference Material (page 9)

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┌─────────────────────┐        ┌─────────────────────┐
│   Define analytical │        │  Select candidate RM │
│ requirement (Note 1)│        │     and obtain       │
│                     │        │    supporting        │
│                     │        │ information (Note 2) │
└─────────────────────┘        └─────────────────────┘
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Reported RM charac-teristics fully match analytical requirement

Limitations but RM is best available and meets minimum requirements

Yes

Yes

No

Supporting evidence concerning quality is satisfactory (Note 3)

No

Limitations but RM is best available and meets minimum requirements

Yes

Yes

No

REFERENCE MATERIAL SUITABLE

RM NOT SUITABLE, SEEK ALTERNATIVE OR DOWN GRADE REQUIREMENT

Notes to Fig. 2:

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

Noite 2: Key characterists 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.

Note 3: See p4 and p5

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