Eur. J. Clin. Chem. Clin. Biochem.
Vol. 29, 1991, pp. 241–246

Reference Materials – A Main Element in a Coherent Reference Measurement System[1])

By *R. Dybkaer*

Klinisk-Kemisk Afdeling, Frederiksberg Hospital, Frederiksberg, Denmark

(Received January 22, 1991)

Summary: The analytical reliability of any measurement procedure requires a specifically designed, coherent reference measurement system, including interrelated measurement procedures and measurement standards such as reference materials. The latter may be characterized by three sets of characteristics. The general characteristics comprise origin, mode of production, physical state and phase, homogeneity, physical form, container, additives, storage conditions, stability, and dangerous properties. The specific characteristics describe molecular composition, analyte, purity, matrix, quantity of interest (including scale), assigned value, and uncertainty of measurement. The additional characteristics concern the way in which values for other characteristics were obtained, the hierarchical position of the material, certificate, instructions for use, and intended function.

The problem areas of reference materials comprise definition of the appropriate analyte, purification, matrix, assignment of values, and nomenclature. The present ambiguous terminology is presented and a systematic structure of descriptive names is proposed (tab. 1).

Introduction

An important *goal of the clinical laboratory sciences* is to produce results of measurement that are clinically relevant, cost-effective and reliable.

The *required reliability* of a given type of quantity should be based on stated clinical needs coupled with a knowledge of biological conditions and analytical possibilities.

Analytical reliability is measured by performance characteristics such as analytical calibration function, analytical sensitivity, analytical specificity, recovery, repeatability standard deviation, reproducibility standard deviation, bias, limit of detection, and limits of determination. They determine the uncertainty of measurement, comprising random and systematic components, determining the interval where the true value of a quantity is likely to lie.

The analytical reliability, together with biological, pathological, and therapeutic factors, determine the *clinical reliability* of a given type of quantity as expressed by the number fraction of correct classifications of the clinical status of persons, based on the results of measurement.

The establishment and maintenance of the stipulated analytical reliability of a measurement procedure require a coherent *reference measurement system* comprising recognized organizations, institutions, laboratories, and industries; scientifically based sets of quantities, units, and reference data; hierarchies of interrelated measurement procedures and measurement standards. All these elements contribute to a quality assurance scheme giving traceability of results to appropriate measurement standards, if possible to the SI base units.

[1]) Based on a lecture given at the Symposium "Reference Methods in Clinical Chemistry – Objectives, Trends, Problems" of the Congress Biochemische Analytik 90, München, May 8, 1990

Measurement standards comprise material measures, measuring instruments, reference materials, and measuring systems intended to define, realize, conserve, or reproduce a unit of measurement or a value of a quantity for transmission to other measuring systems (1). The class of reference materials will be discussed here.

A *reference material* has a value of a measurable quantity sufficiently well established to be used for the calibration of a measuring system, the assessment of a measurement procedure, or for assigning values to materials (1, 2). Some values cannot be given in SI units, but require international units or other arbitrary units together with a specified measurement procedure.

The production and selection of reference materials should be governed by the end-use requirements and are a multidimensional optimization, necessitating a knowledge of essential properties, description, and measurement.

In the following, some important variables characterizing reference materials will be described.

General characteristics

What may be called the general characteristics of reference materials comprise at least ten important properties.

The *origin* of the material may be inorganic, organic, or biological from a stated species.

The *production* may be synthetic, semisynthetic, or from natural material and will often require a purification with a check for impurities. Further treatment may consist of drying, freezing, lyophilization, or spray-drying.

The *physical state* is gas, liquid, or solid, and the material may have one or more inividually homogeneous *phases.*

Homogeneity is a key property of reference materials used in chemistry, and the intra- and inter-sample variability must be stated with regard to the minimum analytical portion, taking any microheterogeneity into account, such as for blood.

The *physical form* is a description of shape, dimensions, number, and amount.

The *container* and/or packaging is often crucial for transport, storage, and sampling. It should be specified as to type, material, closure, and atmosphere.

Additives are often unavoidable, especially for reference materials used in the clinical laboratory. They comprise anticoagulants, antioxidants, antimicrobial agents, stabilizers, and wetting agents. Compound and concentration must be specified.

Storage conditions for the unbroken container must be given, such as temperature, humidity, and light.

Stability of the different components of the material under prescribed conditions is another essential property that must be thoroughly investigated, for example by thermally accelerated degradation. It may have to be re-checked during the life of a batch.

Any inherent *hazard* of the reference material must be prominently displayed, such as toxicity, cancerogeneity, or infectiveness. Any sterilization procedure applied should also be mentioned.

Specific characteristics

The specific characteristics of a reference material, directly concerning any quantity for which a value is given, comprise at least eight properties of the components.

The *molecular composition* of each component should be stated, whether it is completely known ("simple"), or only partially known ("complex").

Any component of special interest, usually the component or so-called *analyte*, must be specified.

The *purity* of the main component in a high-purity material may be stated as mass fraction, volume fraction, amount-of-substance fraction, or number fraction.

The *matrix* or milieu of the material, that is the components that are *not* the analyte, may be virtually none for a pure substance, simple — consisting of one or more components — or complex. Important information concerns any solvent residue, both with regard to stability and the correct record of its quantity value.

The *quantity* for which a value is given must be adequately stated with indications of system, component, and kind of quantity, and with relevant specifications for each.

It is useful to specify the type of *scale* on which the value of the quantity is measured, that is whether it is a nominal, ordinal, difference, or ratio scale. The number of possible values is additional information (3).

The *assigned value* of the quantity considered, in principle, is a product of a numerical value and an appropriate unit. SI units and especially those involving

mole are preferred when relevant, otherwise an international unit should be used rather than other arbitrary units.

The *uncertainty of measurement* should be expressed as an interval within which the true value of the quantity lies with a stated probability. The components of the global uncertainty are caused by inhomogeneity of the material and — during the value-assigning exercise — by instability and variation among laboratories, operators, measuring systems, procedures, and series. They comprise systematic as well as random elements.

Additional characteristics

The additional characteristics of a reference material are no less important than those of the two previous groups.

The *procedures for assessing homogeneity and stability* should be recorded.

The *protocol followed for assigning the value and uncertainty* is important for assessing their reliability. The structure of the protocol, number of participating laboratories, metrological level of measurement procedures, their calibration and control, traceability, reasons for elimination of results, and statistical treatment are valuable pieces of information.

Values obtained by procedures of lower metrological levels may be helpful to the user of the material.

The *geographical recognition* of the reference material should be indicated as international, regional, national, or local.

The *metrological level* is sometimes given by the modifiers primary, secondary, and tertiary, but these terms are not used unambiguously, and a measure of uncertainty is more useful.

The *producer and supplier* of the reference material should be stated.

Any *certificate* should be issued by a recognized measurement standards body.

Detailed *instructions for use* are extremely important for the user to obtain maximum benefit from the material. They comprise the opening of packaging, preparation of sample, techniques for achieving thawing or reconstitution followed by mixing, procedure for obtaining the analytical sample and analytical portion, and sometimes a recommended or mandatory measurement procedure. Also, the required storage conditions after opening the packaging and the storage and stability of remaining material are essential information.

The intended *function* of the reference material should be stated, whether for calibration, internal quality control of accuracy or precision, or external quality assessment through intercomparison schemes. The statistical treatment of the values obtained by the user should be indicated for each of the intended functions.

The *legal role* of the material may be stated as mandatory, optional, or provisional.

Finally, the *price* of one item or smallest set should be given.

Problem areas

At present, major problems in producing and using reference materials occur in the following areas.

The *definition of chemical compounds* that behave as the naturally occurring components of the quantities measured is especially difficult for larger molecules and those with several forms with related functions. New insight in the genetic origin, biochemical production, and biological function of such families of compounds should give solutions to this problem.

The *purification* of synthetic or naturally occurring components without loss of proper reactivity and stability is necessary for the production of high level reference materials. New and gentler isolation procedures are continuously being developed, but macromolecules still offer a challenge.

For some reference materials, a *matrix* mimicking that of the natural samples is necessary, but all possible compositions cannot be produced and the measurement procedure should be reasonably independent of matrix variations.

The assignment of so-called "method-dependent values" to quantities realized by reference materials should be abandonned wherever possible in favour of traceable *reference measurement procedure values* with suitably small uncertainties (4). Inter-laboratory trials with several analytical principles and methods increase the confidence in the accuracy of the value.

It should be general practice to use a given reference material for either *calibration or control* of a given procedure and the respectively appropriate statistics should be applied.

Available reference materials, especially control materials, number many hundreds with thousands of assigned values of varying reliability. There is still a need for high level certified reference materials, such as those issued by the Community Bureau of Reference, EU (5), the National Institute of Standards and

Technology, US (6), the World Health Organization (7), and for intermediate level materials with values assigned by reference measurement procedures.

Finally, the *classification and nomenclature* of reference materials is a problem due to the non-compatibility and partial ambiguity of various systems.

Nomenclature

In view of the many important properties characterizing a material, no reasonably short term can substitute for a full description. The question is whether defined words from current classification systems can be combined into unambiguous and useful terms.

A *standard* in the IUPAC (1965) sense was a high-purity material, graded according to purity in five classes A to E as follows (8).

A atomic-weight standard
B ultimate standard
  virtually Grade A
C primary standard
  commercially available
  mass fraction 1.000 0 ± 0.000 2
D working standard
  commercially available
  mass fraction 1.000 0 ± 0.000 5
E secondary standard
  lower purity than Grade D
  standardized against Grade C

Even grade C is seldom if ever used in clinical laboratories. Later, the word "standard" was used in many terms not necessarily denoting a high purity material, but for example a solution.

Primary as a modifier in the IUPAC (1965) terminology denotes a high-purity material with a mass fraction of 1.000 0 ± 0.000 2 (8), but was later used by IUPAC (1988), to mean thoroughly characterized, stable, homogeneous, and with a stated quantity value and uncertainty (9).

The modifier *secondary* (IUPAC, 1965) meant high-purity material calibrated against a primary standard (8), but has also "degenerated" to indicate a material having a quantity value with a higher uncertainty than the primary standard or containing loosely characterized components. Consequently, the uncertainty of measurement for a given "primary" material now may well be larger than that of another "secondary" material used in another field.

The modifier *clinical* (10) or "clinical laboratory" (11), put before a term containing the word standard, is usually not meant in a "complimentary" way, but as a sign of possible problems due to lack of definition.

The term *matrix* in analytical chemistry denotes all other components in a system than the analyte. In the clinical laboratory sciences the meaning has shifted towards "complex" or "incompletely known", which is unfortunate.

The latest set of authoritative definitions is given in the International Vocabulary of basic and general terms in Metrology (revised draft 1989), abbreviated VIM (1). Here, as mentioned earlier, a *measurement standard* may take the form of a reference material.

VIM further defines the concept *primary measurement standard* as having the highest metrological qualities in a specified field. The term *primary reference material*, thus, would not necessarily indicate a maximum uncertainty on a mass fraction scale or a stable homogeneous material. What is "primary" today could be "poor" to-morrow due to advancing knowledge and technology. A *secondary reference material* is one whose value of a measurable quantity is fixed by comparison with a primary reference material (1).

VIM also defines the concept *reference standard* as usually being of the highest metrological quality available at a given location. This term gives problems with the class of reference materials, as one cannot have the term reference reference material. The VIM *working standard* is calibrated against a reference standard, i.e. the modifier "working" is used differently than in IUPAC's term for a grade D standard defined earlier.

I submit that we have a set of words used ambiguously, namely

- standard
- primary
- secondary
- clinical
- clinical laboratory
- working
- matrix

and a few current terms not yet of doubtful meaning comprising

- reference material
- certified reference material
- calibration
- control

The most general term is "reference material". The word "material" may be replaced by a word for the *state*

- gas
- liquid
- solid

and sometimes with further specification of the *phases*

- solution
- suspension

Examples are

- reference material
  - reference gas
    - reference gas suspension
  - reference liquid
    - reference liquid solution
  - reference solid
    - reference solid solution

It may be useful to indicate the geographical *area of primary recognition* and/or the *issuing organization* and/or whether the material is *certified* or not. This type of information may come before the word "reference" or after the term "material" (or its substitute), as for example

- international reference material
  - international WHO reference material
  - international IFCC reference material
- European reference material
  - European BCR reference material
  - European BCR certified reference material
- national reference material
  - US NIST certified reference material
- local reference material

Information about *origin*, for example inorganic, human, or porcine, can be put before the word "reference", but is usually better given in parentheses after the name or as a second line, as for example

- inorganic reference material
- reference material (porcine)

The same holds for *production* details such as synthetic, natural, or lyophilized, as for example

- natural reference material
- reference material (lyophilized)

An indication of the *matrix* can be given as, for example

- simple reference material
- complex reference material

where "simple" means that all components are known whereas "complex" indicates that some cannot be specified. Alternatively, this specification may be put parenthetically after the name or in a second line.

It may be useful to indicate the *intended use* of the reference material, although many preparations can be used for either calibration or control according to circumstances. One of the two words "calibration" and "control" is often substituted for "reference" or may be put immediately before it or after, as for example,

- calibration reference material
- calibration material
- reference material (for control)

Finally, and most important — perhaps also most controversial — there is the use of the words "primary" and "secondary". The prefaced modifier *primary* is being used with at least three meanings:

- this material has a mass fraction of 1.000 0 ± 0.000 2 of a stated chemical component (IUPAC, 1965) (8), or
- the composition of the material is well known whether it is of high purity or in solution (as propsed by IFCC, 1980) (12), or
- the material was the best available when issued (as VIM defines it) (1); the latter meaning is essentially echoed by NCCLS (1986) (13) and IUPAC (1988) (9).

Tab. 1. Elements and sequential structure of systematic names of reference materials

No	Subject	Example
1	Geographical recognition	international regional (European) national local
2	Issuing authority	WHO BCR NIST
3	Certificate	certified (non-certified)
4	Origin	inorganic human porcine
5	Production	synthetic natural lyophilized
6	Matrix	simple complex
7	Function	calibration control **reference material**
8	State	gas liquid solid
9	Phase	solution suspension
10	Parenthetical or additional	See Nos. 1, 2, 3, 4, 5, 6, 7 above

The modifier *secondary* is also used with several, but related meanings:

- this material has a mass fraction less than 1.0000 and the value can be measured against a primary reference material (IUPAC, 1965) (8), or
- the material can be of any composition and its quantity value is obtained by measurement against a primary reference material (IFCC, 1980 (12); VIM, 1989 (1)).

Here, it is worth noting that measurement is also involved in defining primary materials, at least when characterizing the concentration of impurities.

If the division of reference materials into primary and secondary is thought useful, the VIM definitions are now supported by six international organizations (1), including IFCC and IUPAC. These definitions, however, tell us nothing about the appropriateness of a given preparation for a certain metrological purpose.

It may be more useful to state the best estimate of the value of the quantity of interest together with an uncertainty and probability level. The manner in which they were obtained should appear on an accompanying insert, a report, or a certificate.

As a consequence of this discussion, the name of a reference material may be given according to a flexible format (tab. 1).

The quantity name should be structured according to the IFCC/IUPAC recommendations as adapted for reagents (14) with the reference material itself being the system.

Conclusion

The properties, description, and nomenclature of reference materials are important subjects, meriting a systematic approach, and proper choices are prerequisites for any successful quality assurance scheme.

References

1. International Bureau of Weights and Measures, International Electrotechnical Commission, International Federation of Clinical Chemistry, International Organization for Standardization, International Organization of Legal Metrology, International Union of Pure and Applied Chemistry (1989) International Vocabulary of Basic and General Terms in Metrology. Draft revision, 37 pp. ISO, Geneva.
2. International Organization for Standardization (1981) Terms and Definitions Used in Connection with Reference Materials. ISO Guide 30. 1st ed., 5 pp. ISO, Geneva.
3. Dybkaer, R. & Jørgensen, K. (1989) Measurement, Value, and Scale. Scand. J. Clin. Lab. Invest. *49*, (suppl. 194), 69–76.
4. Stamm, D. (1982) A New Concept for Quality Control of Clinical Laboratory Investigations in the Light of Clinical Requirements and Based on Reference Method Values. J. Clin. Chem. Clin. Biochem. *20*, 817–824.
5. Community Bureau of Reference (1988) BCR Reference Materials. 47 pp. Commission of the European Communities, Brussels.
6. National Institute of Standards and Technology (1990) NIST Standard Reference Materials Catalog 1990–1991. 161 pp. United States Department of Commerce, Gaithersburg, MD.
7. World Health Organization, Expert Committee on Biological Standardization. Technical Report Series. WHO, Geneva.
8. International Union of Pure and Applied Chemistry, Analytical Methods Committee, Analytical Standards Sub-Committee (1965) Sodium Carbonate as a Primary Standard in Acid-Base Titrimetry. The Analyst *90*, 251–255.
9. International Union of Pure and Applied Chemistry, Clinical Chemistry Division, Commission on Automation and Clinical Chemical Techniques & Analytical Chemistry Division, Commission of Analytical Nomenclature (1988) Glossary of Bioanalytical Nomenclature, Part 1. General Terminology; Body Fluids; Enzymology; Immunology. Provisional draft 6, ii + 26 pp.
10. Radin, N. (1967) What Is a Standard? Clin. Chem. *13*, 55–76.
11. Young, D. S. & Mears, T. W. (1968) Measurement and Standard Reference Materials in Clinical Chemistry. Clin. Chem. *14*, 929–943.
12. International Federation of Clinical Chemistry, Scientific Committee, Expert Panel on Nomenclature and Principles of Quality Control in Clinical Chemistry (1980) Approved Recommendation (1979) on Quality Control in Clinical Chemistry. Part 3. Calibration and Control Materials. J. Clin. Chem. Clin. Biochem. *18*, 855–860.
13. National Committee for Clinical Laboratory Standards (1986) Glossary and Guidelines for Immunodiagnostic Procedures, Reagents, and Reference Materials. Approved Guideline. NCCLS *6*, No. 16, i–vii + 477–496.
14. Uldall, A. & Dybkaer, R. (1976) Nomenclature of Reagents. Scand. J. Clin. Lab. Invest. *36*, 305–312.

Dr. René Dybkaer
Department of Clinical Chemistry
Frederiksberg Hospital
Nordre Fasanvej 57
DK-2000 Frederiksberg