Fresenius J Anal Chem (1995) 352:35–39

Fresenius' Journal of
Analytical
Chemistry

Global perspectives for secondary reference materials for analysis of food and related biological samples

G. Venkatesh Iyengar [1], **Wayne R. Wolf** [2]

[1] Research Consultant, NIST Standard Reference Materials Program, Building 235, B125, Gaithersburg, MD 20899, USA
[2] NDL, BHNRC, ARS, USDA, Hyattsville, MD 20782, USA

Received: 27 October 1994/Revised: 28 November 1994/Accepted: 8 December 1994

Abstract. Primary, secondary and tertiary reference materials (RM) play an important role in quality controls of analytical measurements. Logistics of preparation and proper use of primary and secondary RMs are presented. Tertiary (i.e. in-house) control materials are useful as substitutes in the absence of recognized primary or secondary RMs. The lack of interdisciplinary interaction during development of RMs (e.g. in specific areas such as foods), has an important impact on limiting the usefulness of certain types of RMs. The abundance of RMs in some countries and regions appears to have little effect on the existing paucity in RMs in other regions, and the underlying causes are outlined. The ability of a laboratory to produce good quality in-house RMs traceable to recognized primary or secondary RMs is a direct measure of its quest for reliable analytical data. Therefore many laboratories should be encouraged to engage in secondary and tertiary RM activities designed to answer specific measurement problems. In this context, assistance (e.g. practical training opportunities) in identifying simple methods of analyses for their efficacy in determining specific analytes is a source of help that can be extended to countries experiencing limitations in laboratory instrumentation.

Introduction

Today's demands for increased emphasis on accuracy and validation of analytical data is exemplified in the rapidly growing acceptance of ISO 9000 quality guidelines, which have a strong foundation of "traceability" to certified reference materials (CRM) for acceptance of analytical measurements. Considerable activity throughout the world is beginning to provide the often complex and demanding standard methods to meet these measurement demands. These demands also call for increased efforts in proper understanding of the roles played by both primary (i.e. certified by recognized agencies) and "suitable" secondary reference materials (RM). An important function of a primary RM is in the validation of the capabilities of analytical techniques to obtain accurate results, including development of sophisticated and innovative analytical approaches to enhance the excellence of the underlying metrology to obtain results of high analytical accuracy.

On the other hand, a secondary RM is usually a "work-horse" version of a primary RM, designed to serve the practical and day-to-day quality control needs of analytical measurements. Thus, the use of a secondary RM ensures consistency of the desired level of analytical accuracy of an investigation. In some cases, the function of a secondary RM may be limited to providing quality assurance for a few target analytes in field studies. While the usefulness of primary RMs in methods development and validation is a broadly discussed subject, the role of a secondary RM is often inconclusively understood and interpretations are blurred. This presentation deals with the aims and scopes, cost considerations, and proper applications of secondary RMs.

Classification of reference materials

The terminologies used to identify various classes of RMs considered in this report are (i) Primary Standards, (ii) Primary RMs, (iii) Secondary RMs, and (iv) Tertiary RMs.

Primary standards and the primary RMs are (generally) produced and certified by institutions with a proven record of analytical insight and experience. Both primary standards and primary RMs mentioned here comply with the general definition of certified reference materials (CRMs) as defined by the International Standards Organization [1]. However, "primary standards" are pure chemicals supplied for the purpose of calibration of an instrument.

Primary RMs represent carefully chosen matrices (natural and spiked) designed to test the performance of a new analytical technique. Thus, they directly address the question of analytical excellence. These primary RMs are generally internationally recognized with an undisputed level of analytical confidence.

Secondary RMs represent a broad group of analytical quality control materials meant for frequent use, and may

Correspondence to: G.V. Iyengar

Table 1. Logistics and some features relevant to primary and secondary RMs

Parameter	Primary (certified) reference material	Secondary reference material
Goal	High accuracy; demonstrate metrological excellence in carefully chosen set of representative matrices	Desired accuracy/problem oriented; designed to solve specific matrix problems and analyte concentrations
Requirements	Proven measurement capability evidenced by exceptional analytical insight and expertise; access to multiple analytical techniques of high credibility; development of stringent preparatory conditions to satisfy broad-based use	Established analytical expertise and thorough understanding of the matrix related performance of the methods chosen for the investigation; flexible preparatory conditions possible to accomodate analyte and matrix specific needs
Proper use	Testing performance of new analytical techniques and as changes are introduced to existing methods; generally used in small quantities; traceability in chemical metrology; lead analytical quality control material in the development of secondary RMs/methods validation	As an analytical quality control material for frequent check of performance of analytical techniques/intercomparison runs, particularly to study matrix effects; less limitations on quantities used during analysis, hence the advantage of matching size of the QC material to that of real sample, if necessary
Cost	Expensive. More than the cost aspect, the infra-structure requirements for producing primary RMs are very complex and sophisticated; hence confined to a few Centers	Moderate; importantly, the ability to participate in the preparation of secondary RMs and their characterization enhances the integrity of the investigations that follow

have numerous origins. These materials offer the best opportunity to address analytical quality assurance aspects at the regional level, designed to address practical problems. Assignment of "best" values for these materials should be traceable to primary certified materials.

The tertiary class of RMs represent various types of in-house pools of quality control materials of selected matrices produced for use within a laboratory or for utilization by a group of investigators. These can be prepared either in small quantities for a specific project, or in large quantities for frequent use as daily quality control samples.

The basic features of primary and secondary RMs are compared in Table 1. As highlighted in Table 1, the role of primary (i.e. certified) RMs in a measurement system is distinctly different in comparison with that of secondary RMs. For an extensive discussion on the impact of certified RMs on measurement processes, the reader should consult Ref. [2].

Primary, secondary and tertiary RMs

Primary RMs represent matrices with (i) natural concentration levels (including naturally high accumulation in some matrices), (ii) metabolically enriched levels through internal spiking of a biological system, or in some cases, (iii) the concentration of a given analyte merely elevated by external spiking. Examples of the above mentioned types of matrices are (i) non-fat milk powder (NIST SRM 1549) and bovine liver (NIST SRM 1577) for endogenously elevated level of Cu, (ii) a Japanese rice standard (NIES CRM 10c) containing high Cd levels as a result of contaminated paddy fields, and (iii) toxic metals in freeze-dried urine (NIST SRM 2670) prepared by external spiking to raise the levels of Cd, Cr, Hg, Pb and Se.

The usefulness of the secondary RMs is best seen at the national level for resolving analytical problems of regional interest. They need to be produced in fairly large quantities by recognized national laboratories for frequent use by participating analysts. The requirement is that these RMs should be reasonably well characterized to meet the purpose of the regional problem, and the quality profile should be traceable to a primary reference material of a comparable matrix (i.e. appropriate primary RMs should be used for an accuracy check during chemical characterization of the secondary RM). Certain materials produced by commercial sources, but traceable to a certified RM, also qualify as secondary RMs.

There have been instances where secondary types of RMs have appeared ahead of primary RMs for a host of reasons. One reason for such an occurrence is the lack of two or more suitable analytical techniques to facilitate the thorough investigation required for certification. In some cases, these situations have provided the impetus for methodological development, leading to the issuing of primary RMs. The development of mixed diet RMs is a good example. Building upon knowledge gained in development of an initial pool of mixed diet material (RM 8431), a program initiated by the United States Department of Agriculture (USDA) [3], a total diet RM of the primary type (NIST SRM 1548) was issued by the National Institute of Standards and Technology (NIST), with the help of USDA and the United States Food and Drug Administration (USFDA) [4–6].

Tertiary or in-house RMs are particularly crucial in context of some laboratories deprived of access to a regular supply of secondary or primary RMs but who are committed to maintaining control of the analytical quality of their results. In some cases, tertiary RMs serve to provide specialized matrices, when even a secondary type of RM is not available. In both cases, where possible, at

least a comparable secondary RM should be used to establish the analytical validity of the in-house RM. These specialized matrix types of materials have the advantage of closely resembling the real analytical sample. They are also useful when some gross matrix effects have to be tested; e.g. extraction of an analyte in the presence of differing matrix compositions, e.g. fat, fiber and/or phytate. Check samples produced by commercial sources for specific uses (e.g. calibrants for a glucometer), although not always tested against a recognized primary or secondary RM, may qualify as tertiary RM.

One example of a tertiary RM is the human milk pool which was specially developed for the World Health Organization (WHO) and the International Atomic Energy Agency (IAEA) project to study the chemical composition of breast milk [7]. In the absence of a recognized primary or secondary human milk RM, this QC material served as the analytical quality control link between the reference laboratories and the back-up laboratories. The IAEA RM A-11 (whole milk powder) served as the traceability link in this case. A second example concerns the recent initial characterization of soya-based powdered infant formula and Cocoa Powder by the USFDA to provide suitable in-house RMs to maintain frequent monitoring of the performance of methods used in its field laboratories [8]. A recent AOAC publication [9] provides information useful in understanding the role of matrix composition and preparation of representative in-house food RMs.

Reference materials for food analysis

Commonly used RMs in food and nutrition laboratories are those characterized for (i) nutritional components, (ii) environmental chemicals, (iii) microbial contaminations, and (iv) environmental radioactivity.

Nutritional components would include the proximates, and organic and inorganic nutrients such as vitamins, minerals and trace elements. Environmental chemicals include among others, organic contaminants such as chlorinated organic compounds, organic pesticides, polycyclic aromatic hydrocarbons, metallo-organic compounds, toxic metals and finally sulphur and nitrogen oxides. Microbial contaminants include natural toxins, among others. Radioactive contamination of foods is linked to atomic bomb test residues or the Chernobyl type of accident (where e.g. Cs-137 is released). Further, it would be desirable to have each group of analytes certified at natural concentrations (which may be high or low with respect to nutritional levels), intermediate concentration levels (in some cases representing transition levels from nutritional to toxic), and high concentrations (aimed primarily at toxicity). In this context, the concept of secondary RMs becomes very useful. For further discussion on matrix effects due to a major food component such as fat, refer to the paper by Anderson et al. (this issue).

In dealing with a good quality RM, efforts necessary for the preparation of the material and associated cost of analysis for certification, require careful evaluation. In addition, the groups of analytes determined, and the quantity of RM required per determination, play a significant role. From a user's point of view, the cost of a RM is relative to the analytes certified and the quantity of RM supplied per unit. Since it is impractical to generate a single certificate of analysis to include all the components of possible interest, alternate approaches are needed. To minimize the preparatory efforts, carefully chosen representative matrices should be prepared in large quantities and utilized in batches for certification of specific groups of analytes. For example, NIST RMs for trace elements (NIST RM 8415) and cholesterol (NIST SRM 1845), belong to the same pool of whole egg powder. Depending upon the analytical requirement, unit size/packing can be varied.

Present status of food RMs

Taking a wholistic view of RMs and their role, with few exceptions it seems that a significant portion of the RM concept is still in its gestational phase in most areas of the world. This becomes obvious when reviewing the present status of RMs in the life sciences areas. While considerable progress is seen for some types of RMs (e.g. food), a closer examination reveals a lack of coordination between different disciplines.

RMs for inorganic constituents: Assessing the progress in trace element chemistry of foods, Hertz and Wolf [10] commented in 1985 that the "state of the practice lags behind state of the art", meaning efforts were lacking to implement the existing technology to address real world problems in inorganic analysis of foods. Education and appropriate training in analytical chemistry still hold the key for solution to the analytical quality control (AQC) problems. The impact of this factor is particularly acute in several developing countries.

In assessing the progress related to inorganic RMs, compiling a list of food RMs characterized for a typical analyte such as Zn revealed over 60 sources. When the RMs are classified into groups of similar matrices, they were found to represent, barring a few exceptions, many repetitive profiles. Only a handful of these available RMs are characterized for components such as proximates and major minerals, which are basic for the AQC requirements of a food analysis laboratory. It would seem that a major part of the AQC objectives achieved for a set of inorganic analytes by using all of the 60+ RMs, could have been accomplished by the use of a small number of selected RMs. The preceding example serves to highlight three aspects:

(i) the absence of interdisciplinary interaction during development of RMs, leading to diminished overall usefulness of a given RM;

(ii) the abundance of RMs in some countries appears to have little effect on the existing paucity in other regions which are still craving for such AQC-tools [11], and

(iii) continued failure to recognize these shortcomings will only extend the gestation period for world-wide improvement in AQC.

The preceding example points to consolidation in future inorganic RM certification efforts by identifying such analytes that closely reflect user needs. Paired matrices such as soya flour and milk powder (fat and non-fat), spinach and potato powder, egg powder and animal

muscle, oyster and fish tissue, etc., would represent a broad enough range of analytes (proximates, nutrient, contaminants, fiber, fat etc.) commonly sought for in food and nutrition RMs.

RMS for organic constituents: In the area of organic analysis, procedures for analytical quality assurance for many constituents are still evolving. There is ample awareness among investigators recognizing the need for generating reliable results, but this perception has not translated into action other than the recognition of inconsistencies stemming from intercomparison trials. This is not swiftly followed up by identification of the sources of discrepancy, initiation of the remedy, and subsequent AQC exercises. One obvious reason for this slow progress is the paucity of funding for basic research in analytical methodology, thus shifting a major fraction of the developmental work to a few institutions such as those dealing with RM programs.

The factors mentioned above are only partially responsible for the lack of rapid progress in developing a wide variety of urgently needed organic RMs. There are of course genuine methodological and technical hurdles, by far the most challenging step being preparation of a suitable natural matrix while retaining the compositional integrity of the organic material, without excessive cost for extended preservation.

Established agencies such as the European Community Measurement and Testing Program (MTP, Belgium), NIST (USA), International Atomic Energy Agency (IAEA Austria), National Institute of Environmental Studies (NIES Japan), and The National Research Council of Canada (NRC, Canada) have developed several certified RMs ranging from simple solutions for calibration of analytical instruments to complex natural matrix materials. Some of these are fortified, suitable for validation of methods adopted for nutritional and environmental biomonitoring and related programs.

For example, the NIST Mussel Tissue (Mytilus edulis; NIST SRM 1974) is certified for Anthracene, Benzo(b) fluoranthene, Benzo(ghi)perylene, Benzo(a)pyrene, Indeno(1,2,3-cd)=pyrene, Fluoranthene, Pyrene, Perylene and Phenanthrene. It also contains information values for a number of other PAHs, PCBs and chlorinated pesticides. Similarly, CRMs of organics in marine sediments (NIST SRM 1941) and Cod Liver Oil (NIST SRM 1588), PCBs in Human Serum (NIST SRM 1589) are available. Very recently, frozen whale blubber (NIST SRM 1945) has been developed by NIST for use as RM for organic and inorganic contaminants including methyl mercury. This SRM has been analyzed for 30 polychlorinated biphenyl congeners and 16 chlorinated pesticides. Similarly, IAEA has released the shrimp homogenate (MA-A-3/0C) and lyophilized fish tissue (MA-B-3/0C) for chlorinated hydrocarbons. The European Community (BCR Belgium) has CRMs for pesticides in milk powder (CRMs BCR-150, BCR-51, BCR-152) and pork fat (CRM BCR-430), and for aflatoxins in milk powder (CRMs BCR-282, BCR-283, BCR-284, BCR-285). Concerning organo-metallic contaminants, besides NIST SRM 1945 (whale blubber), which is certified for methyl mercury, fish tissue from the NIES, Japan (NIES-11) has been certified for total Sn, tributyl and triphenyl Sn. The NRC, Canada has issued Dogfish Liver (NRCC-DOLT-1), Dogfish muscle (NRCC-DORM-1), Lobster Hepatopancreas (NRCC-TORT-1) and Non-defatted Lobster Hepatopancreas (NRCC-LUTS-1) certified for methyl mercury.

Global needs for secondary RMs

In our opinion, it is not possible to offer a quantitative estimation for the global requirement of secondary RMs. However, it is possible to get a fair idea of the existing potential for overall demand. First of all, the growing interest by many countries to comply with ISO 9000 requirements augments the scope for RM projects. This is also reflected when browsing through past proceedings of the BERM meetings [12]; over a span of 10 years a host of new RMs have emerged in the areas of biology, food, and environment. The same source also reveals that these RM activities are taking place in a select group of countries, mainly the USA, Canada, Japan, China, and a few in Europe. In this context, it should be recognized that secondary RMs produced for use in one country/region may not necessarily be the best solution for the matrix problems prevailing in other countries. Therefore, even a modest effort by a few more countries for preparing secondary RMs, would generate a great deal of activities.

In identifying the relevant types of secondary RMs for a given country, obviously the local needs (environment, soil, food habits, trade priorities, etc.) are the determining factors. However, producing purified water and using processed foods such as milk powder (whole and non-fat), soya products that are available as whole and defatted commodities, and egg powder would make a good start since they would be available in a convenient form requiring minimum handling. Hay or cabbage powder would provide a good working QC material for environmental purposes. Of more practical concern to developing countries is the production of microbiological RMs. However, methodological problems with these types of RMs are still very formidable, and even the preparation of a homogeneous matrix pool is technically difficult, and microbial viability (hence stability of the microbial counts) is difficult to sustain if conditions are not stringently controlled. Hence, problems with the development of these types of materials will persist for some time.

Practical hints for meeting global needs of secondary RMs

1. Existing RMs alone cannot meet the global needs of secondary RMs, for several reasons alluded to above. Acquisition of secondary RMs (by whatever means) from external sources, is at best only a temporary solution.
2. As a matter of self sufficiency, as many countries as possible must learn to prepare their own secondary and in-house RMs, to better suit their analytical requirements. This would also mitigate the often mentioned cost and currency related difficulties as impediments. The ability of a laboratory to produce good quality in-house RMs is a direct measure of its quest for reliable analytical data. However, all such efforts should be backed by analysis of

appropriate primary RMs as a measure of accuracy check and validation of the method applied.

3. It would be prudent to consolidate the process of information dissemination about RMs, i.e. improved access to published information (e.g. existing RMs, their merits and demerits, and gaps in RM profiles) for RM-deficient countries. In this context, efforts initiated by the IAEA (RM Compilation), SRM databases (e.g. COMAR/REMCO/ISO) and the United Nation's Environmental Protection Agency's (UNEP) Harmonization of Environmental Measurements (HEM) program, are all well suited.

4. Identification of simple methods of analysis for their efficacy in determining specific analytes is yet another source of help for countries experiencing limitations in laboratory instrumentation. Some guidance in this context can be seen in a recent report by the World Health Organization [13].

5. Providing practical training for analysts in countries less exposed to the problems of preparing RMs would be the most effective way for meeting needs. Regional programs by agencies such as the IAEA to provide technical assistance and practical training are crucial in reaching the goal of supplying needed RMs.

6. One or two laboratories in a region should be identified to strengthen the technical base so that they can assume the role of reference laboratories. These reference laboratories should be able to evaluate the performance of other laboratories in the region, functioning as local training centers.

References

1. ISO Guide 30 (1981) ISO, Geneva, Switzerland
2. Taylor JK (1985) Handbook for SRM users. NBS Special Publication 260-100, NBS, Gaithersburg, MD
3. Miller-Ihli NJ, Wolf WR (1986) Anal Chem 58:3225–30
4. Iyengar GV, Tanner JT, Wolf WR, Zeisler R (1987) Sci Total Environ 61:235–252
5. Wolf WR, Iyengar GV, Tanner JT (1990) Fresenius J Anal Chem 338:473–475
6. Tanner JT, Iyengar GV, Wolf WR (1990) Fresenius J Anal Chem 338:438–440
7. Parr RM, DeMaeyer EM, Iyengar GV, Byrne AR, Kirkbright GF, Schoech G, Niinistö L, Pineda O, Vis HL, Hofvander Y, Omololu A (1991) Biol Trace Elem Res 29:51–75
8. Anderson D, Cunningham W (1995) American Nuclear Society Transactions (in press)
9. Sullivan DM, Carpenter DL (eds) (1993) In: Methods of analysis for nutrition labeling. AOAC, Arlington, VA
10. Hertz H, Wolf WR (1985) In: Hertz H, Wolf WR (eds) Biological and environmental reference materials. Wiley, New York, pp 301–310
11. Iyengar GV (1993) Fresenius J Anal Chem 345:107–108
12. Proceedings of the International Symposia on Biological and Environmental Reference Materials, Ref 10 (for BERM-1). Fresenius J Anal Chem 326: No 7 (1987); 332: No 6 (1988); 338: No 4 (1990); 345: No 2–4 (1993); see also 348: No 7 (1994), for BERM-2 to 5, respectively
13. Iyengar GV (1995) In: Trace elements in human nutrition and health, Chapter 3. WHO Report, Geneva, Switzerland (in press)