

REFERENCE MATERIALS FOR TRACE ANALYSIS

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Abstract - An account is presented on several aspects of a reference material before it can be recommended for use - especially in trace analytical work. The material should meet with certain basic requirements, e.g. it should be stable, low in contamination, homogeneous in composition, be available in sufficient amount etc. Guidelines are presented while evaluating the results obtained by participating laboratories in the certification procedure. The article includes sources for obtaining reference materials including environmental and energy related materials as well as biological and botanical materials.

CONSIDERATIONS FOR THE USE AND NEEDS OF REFERENCE MATERIALS

In any analysis it is always mandatory to question the accuracy of the result. The analyst performing determinations of trace contents in various materials must therefore develop and maintain what one may call a healthy scepticism concerning the results obtained by his laboratory. If this scepticism develops quality assurance, one of the keywords nowadays in scientific and technological performances, has made a major step forward in the analytical laboratory. The important word in this paragraph is obviously "accuracy". There is however some confusion between an accurate analysis and an accurate value. Therefore Cali et al. (1) at a conference devoted to accuracy in trace analysis, some years ago offered the following formal definition: "An accurate measurement system is one that produces precise numerical values of the property under test or analysis that are free of, or corrected for, all known systematic errors. Such values are also related to the "true value" of the property under test or analysis". We all know that realizing such an accurate measurement system and obtaining so called "accurate values", is not an easy task in trace analysis of various materials. It implies among others the collection of a contamination free and representative sample, the selection of an appropriate analytical method which is free of systematic errors, sufficiently sensitive and precise for the problem under consideration and a careful, error-free execution of the analysis.

That this was not always achieved may be illustrated by the determination of the normal level of Al in serum or plasma (Fig. 1) by 18 laboratories (2). Nearly all these data were published during the last 5 years. Even larger variations were obtained for Cr in the NBS Standard Reference Material SRM-1577, bovine liver ($< 0.005-3.5 \mu\text{g/g}$) (3). Evidence has since accumulated that an important source of error was the unexpected contamination of samples during collection or manipulation in the laboratory. A wealth of other examples illustrating the lack of agreement, and thus of accuracy in trace analysis can be found in reports on inter-comparisons, round robin analyses and certification attempts.

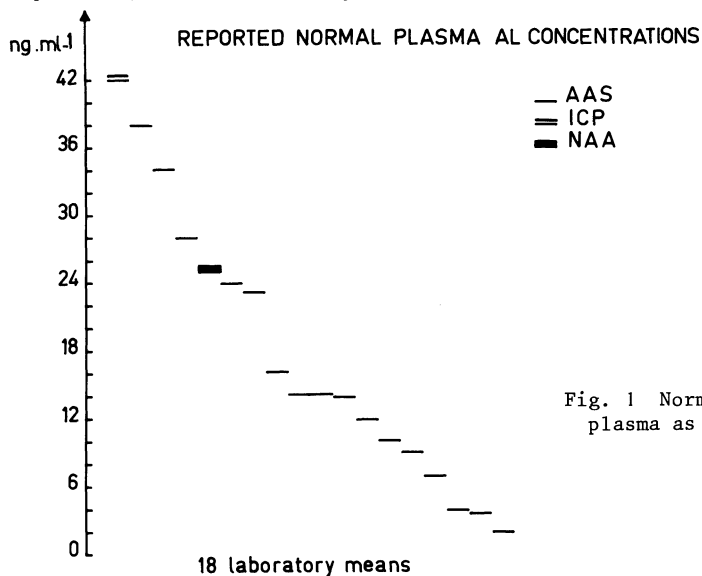


Fig. 1 Normal Al-concentrations in serum or plasma as determined by 18 laboratories (2).

Immediately the question arises, how then, in practice, can measurement accuracy be achieved in a laboratory. One of the most direct ways is the application of reference methods or the use of reference materials. Such reference materials are usually carefully studied and well-characterized materials, in terms of concentrations of certain trace components, and homogeneity and stability, which are produced in sufficient quantity so as to be continually available. Reference materials are in fact keystones in a quality assurance plan.

They find several applications. They may be used to calibrate instruments, or better to verify the accuracy and the precision of the entire analytical system used in the laboratory. While replicate analyses of a reference material indicate that the precision obtained by the system is good enough, the deviation of the mean analytical value from the "true" or certified value will be a measure of the systematic bias of the system. This strategy can improve the quality of the measurements. The availability of a reference material together with the certification report enables the analyst every time he feels the need to match the performance of his laboratory with the performance of the certifying laboratories. Each time he can prove to himself that his methods when applied to an unknown but similar sample arrive at the right results. By this strategy he sets an important step towards quality assurance of his laboratory. He can verify the performance of his instruments and analytical methods, each time when there is a risk of bias, for example due to a possible interference.

More and more emphasis is laid upon the true or accurate concentration of trace constituents, rather than upon a relative agreement between methods. In fact when only reference is made towards a common, not necessarily accurate base, problems arise when results must be compared across different technical or scientific fields. An obvious example is the study of the pathways of a component through the different parts of the environment. A material balance can only be made when the obtained concentrations in the different matrices are close to the true value. The same is true for the assessment of production processes of for example precious metals from raw material towards endproduct. Also legalization for quality assurance in food and drug industry, in pollution abatement, etc. are less complicated when the measurements are made on the basis of accuracy, so that only the concentration must be specified and not the method for the determination. The greatest argument in favor of accurate measurements and thus of reference materials with true values is compatibility. The specified true value can be found, within the specified uncertainty with all appropriate methods of analysis. In analytical chemistry the reference material provides a real matrix sample material for the evaluation of a newly developed technique or procedure. When used in intercomparison studies it can permit the evaluation of the equivalency between different analytical techniques.

The knowledge of trace and ultra-trace concentrations of inorganic or organic constituents has become important in the last decades in a large variety of materials from different origins and for wide-spread use in society. This resulted in the development of very sophisticated analytical equipment, but also implies the need for a wide variety of reference materials, studied or certified for the very small amounts of elements or components present. These include ores and minerals but also high purity metals in which the concentration of certain trace components may determine their quality. In cosmo- and geochemistry and in agricultural investigation the importance of trace elements is more and more being realized. In the quality improvement of biomedical, biological and botanical analyses the relatively recent issuing of reference materials has played a very significant role. Also in environmental energy related pollution and industrial health studies the occurrence of a number of reference materials has already partly satisfied an important need. The extreme success of the NBS 1632 fly ash SRM, soon after exhaustion followed by the issuing of a new fly ash material 1632a proves this point. There is however still an urgent need for additional reference materials. In fact in this relatively new field existing methods were applied to new matrices by laboratories used to handle completely different types of materials. As Skogerboe (4) claims laboratories concerned with analysis of environmental samples are perhaps most susceptible to the analytical inaccuracy problem. This derives from the fact that a large number of environmental samples delivered for analysis have been collected by individuals from other disciplines who have little or no experience with taking representative samples and avoiding contamination.

An important question is which elements or compounds should be certified and which not. This question is for biological, botanical, agricultural and environmental samples, at the present state of our knowledge, a very difficult one. In environmental investigations pollutants should in fact not only be ranked on the basis of their potential toxicity. Most elements may in one sense or another be important. Spyrou (5) for example compiled a table of elements and compounds of interest in environmental work, whether as indicator, as an element of environmental interest or as an element of toxicological interest. It appears that nearly all elements have for some reason a potential interest. Therefore the safest approach is to certify the reference materials for as many elements or constituents as possible.

REQUIREMENTS FOR A REFERENCE MATERIAL

A number of national and international bodies or organizations have produced and issued reference materials. They are usually carefully studied materials, for which particular trace elements are specified in some way. This specification can be presented under the name of certified value, non-certified value, indicative value, information value, recommended value, probable value, consensus value, overall mean, etc. The credibility associated with such a reference material is of course directly related to the degree of confidence placed in the certifying organization.

In the United States reference materials as produced and certified by the National Bureau of Standards are called Standard Reference Materials (SRM). Certified Reference Materials (CRM) are prepared at concentrations within $\pm 1\%$ relative of existing gaseous Standard Reference Materials and are analyzed by comparison to SRM's. The IUPAC Commission I.4 has however recommended to use "Reference Materials" as general class name for well characterized materials, where Certified Reference Materials (CRM) can be compared to the NBS SRM's and have certified values while Calibration Test Reference Materials (CTRM) include reference materials which are not certified. Other organizations such as BCR of the Commission of the European Communities simply name their certified materials Reference Materials (RM), while IAEA produces Certified Reference Materials (CRM) as well as Reference Materials (RM) for application in nuclear, environmental and biomedical investigations. Other classes of reference materials are called Secondary Reference Materials, Commercial Reference Materials, etc.

A number of important requirements are however to be fulfilled before a material becomes useful as reference material in a quality assurance program. The primary requirement is of course the representativeness for a certain well-defined type of sample of interest. Ideally the reference material should have the same or nearly the same matrix, and it should match the samples to be analyzed with respect to levels of trace elements. For biological samples these levels should be the natural ones, that is not be contaminated during handling and preparation. If possible also the speciation, that is valency and binding, should be the same as in the real matrix. This requirement immediately implies the need of a large variety of reference materials in the environmental, biological and biomedical fields.

The second requirement asks for homogeneity. It is essential that every subportion of a given lot be the same within the overall uncertainty limits provided on the certificate. Before a certification process or even an interlaboratory comparison is started the homogeneity should be studied at various levels of sample intake. The analytical techniques to be used must have a high precision (reproducibility), and are therefore often chosen between the purely instrumental ones such as neutron activation analysis, X-ray fluorescence or another spectrometric technique. It is often not necessary to investigate all the trace components to be analyzed, but trace elements preferentially associated with different phases of the matrix should be selected for this homogeneity test. Examples of these different phases are the inorganic and the organic fractions of coal, soil, plant material, etc. or the large and small particle fraction in fly ash and in urban particulates. It is therefore wise also to check on the homogeneity of some matrix constituents.

Recently Frauerwieser (6) discussed the possible errors due to inhomogeneity when an environmental particulate material such as urban particulate matter is to be used as reference material or in collaborative tests. It is assumed that all particles are spherical and uniform in density and that it consists of a two component mixture. With all these oversimplifications it appears that for a sample intake of 100 mg, a 25-50 μm grain size and a density of 2 g. cm^{-3} only 0.75×10^6 particles are sampled, which might introduce a sampling error of 1.5% for the lead concentration being about 0.6%. The user should also be warned for inhomogeneity within a subsample (e.g. a bottle) due to settling of particles of different dimensions or density as they occur in environmental samples. Guzzi et al. (7) gave a method to calculate the degree of inhomogeneity from practical measurements on solid samples. They define the degree of inhomogeneity as :

$$s_{\text{inh}} = \sqrt{s_{\text{obs}}^2 - s_{\text{R}}^2} \approx \sqrt{s_{\text{B}}^2 + s_{\text{W}}^2}$$

s_{B}^2 = variance between bottles ;
 s_{W}^2 = variance within bottles ;
 s_{obs}^2 = the observed scatter ;
 s_{R}^2 = the variance due to the analytical method.

Experimentally often degrees of inhomogeneity of 1 to 3% are found for solid environmental pollution and botanical samples. The minimum sample size (50-250 mg) is however often too large for some very sensitive purely instrumental techniques.

Also stability of a reference material is a matter of concern. If the material changes in time, for example due to evaporation or chemical reactions under the influence of temperature or light, due to precipitation, due to bacteriological activity, due to interaction with the container in which it is stored, etc., the true values may ultimately have changed, or at least the speciation or part of the matrix will be altered. Obviously this is especially a matter of concern for organic or volatile (mercury, organometallics) components and with solutions. Therefore the stability should be tested under conditions which accelerate the conditions which might occur in the laboratory. It is strongly advised that reference materials, whose stability is not infinite under normal laboratory conditions are provided with an expiration date. During storage certain materials may absorb water, preferentially the upper layers of the bottle. Therefore the certificate must give instructions for handling and storage. In order to avoid losses of volatile or unstable compounds during drying it is becoming common practice to determine the dry weight on a separate sample. As a matter of fact, a sufficiently large amount of material must be available, so that it can serve as a reference material during at least five to ten years, if the stability is high.

The certificate should thus indicate :

- The origin and the source of the sample as well as a short description of the preparation and processing.
- The matrix composition.
- The homogeneity limit. This is the minimum size of a representative subsample.
- The stability, with possibly an expiration date.
- The storage, drying and handling instructions.
- The analysis procedures applied for certification, together with the data obtained in the certification campaign, and the statistical treatment applied to the data obtained.
- The certified values all provided with conservatively stated uncertainties.
- If possible information or indicative values for not certified components.
- Any other information or instruction that might be helpful to the user.

CERTIFICATION PROCEDURES

If there is a need for certifications of a certain material a sufficient amount of material must be assembled and the necessary precautions are taken to assure the production of a stable material, low in contamination and homogeneous in composition.

Up to a few years ago important mistakes have been made, also by NBS, in the preparation of biological standards. A typical example where such significant contaminations has occurred during the preparation, that the final reference material is not representative anymore, is the NBS-SRM 1577 bovine liver. It was prepared with stainless steel tools, resulting in a 10 to 10³ fold increase in Co and Cr contamination. The analyst using this reference material to test his analytical procedure will be confronted with a large lack of sensitivity when analyzing real-world samples. Another example is the Brewers yeast, NBS SRM 1569, certified for Cr, in order to solve the controversy on the possible Cr-losses during the drying and ashing steps. De Goeij et al. (8) found that it contained 31% immediately soluble Cr that could be distilled as CrOCl₂ while the other 69% were insoluble and bound as silicate. Therefore nowadays the preparation of SRM's is performed under rigorously controlled conditions, including class 100 facilities for packaging.

The other alternative, i.e. the preparation of artificial reference materials, even when using the highest purity materials and the highest standards of contamination control during preparation does mostly not lead to a representative reference material. An example of an almost useless certified reference material is the Air-3/1 Simulated Air Filter prepared by IAEA (9). Aliquots of solutions were spotted on filter papers and dried under an infra-red lamp. An attempt was made to simulate the concentration ratios of the elements as they occur in actual atmospheric particulate matter. However some large mistakes were made. The gold content is much too high, with a Au/Fe ratio of 5.5×10^{-3} where this is in the order of 10^{-5} in actual samples. As a result of this instrumental neutron activation of this reference material will yield basically a ¹⁹⁸Au gamma spectrum a few days after irradiation. In general the concentrations of the matrix elements added (Al, Ca, Mg, Na, K) are much to low as compared to those of the heavy metals. Since there is no real matrix available the major elements, C, Si, H, S are missing. The overwhelming drawback of this reference material is probably the entirely different chemical and physical properties of it as compared to particulate matter collected on top and partly inside a filter material. For instrumental techniques such as X-ray fluorescence, which analyse the particulates on the filter itself, this material is completely useless.

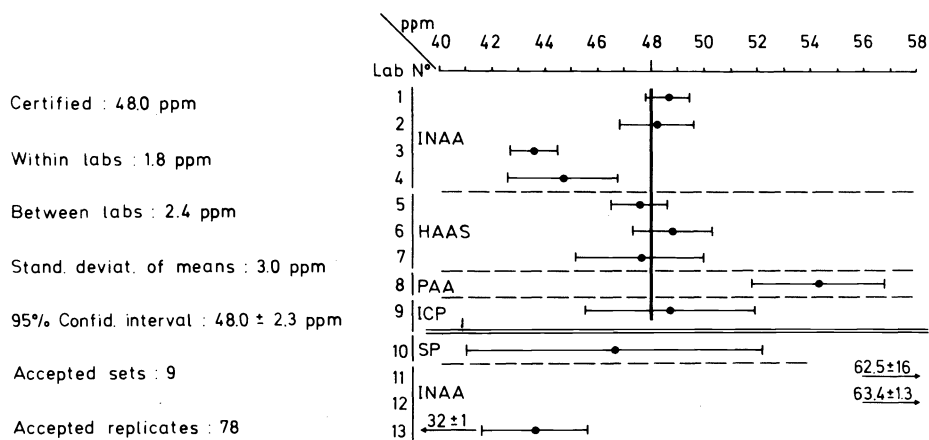
Once a sufficient amount of real material is assembled and fractionated in subsamples, the representativity is established, the stability and the homogeneity are controlled, the most difficult task is still to come, namely the certification itself, that is obtain concentration data which approach as closely as possible the "true value", together with uncertainty limits. If possible NBS will use three modes of measurement to assure the accuracy of the SRM's. These are (a) definitive methods, (b) reference methods and finally (c) two or more

independent and reliable methods. In a definitive method all major significant parameters can be related by direct or a solid chain of evidence to the base or derived units of the SI (1). Examples applicable in trace and ultra-trace analysis are isotope-dilution mass spectrometry and calorimetry, but often this is not possible. Reference methods are, for the variety of materials required in trace analyses, simply not available. Thus the certifying agency must often rely on independent laboratories, using the same or different methods. The laboratories taking part in the analytical campaign should be carefully selected, were criteria are the reputation of the laboratory, its experience in the field of trace elements, its familiarity with the matrix to be investigated and the availability of the required analytical technique. The certifying organization must assure that a wide range of reliable independent methods is covered (typically 3 methods) each used by at least three independent laboratories. Independent means that the basic principles used for the analysis are entirely different, so that possible interferences or other systematic errors can reasonable be expected to be different. In practice many reference materials are certified in this way by NBS. Cali et al. (1) mention the example of Hg in water (SRM 1642) to illustrate what can be achieved by this method. Three completely different techniques were used namely isotope dilution spark-source mass (SSMS), atomic absorption (AAS) and neutron activation (NAA). The results of 10 replicate determinations yielded the following mean values : SSMS : 1.187 ± 0.023 ng/ml ; AAS : 1.17 ± 0.029 ng/ml ; NAA : 1.14 ± 0.08 ng/ml. Certified value : 1.18 ± 0.04 ng/ml.

This is also the procedure as it is applied by BCR, where the laboratories are chosen from all the EEC member countries. The laboratory which participates in such a certification should consider this analysis as a very special one, to be performed with all possible care, and not have it performed by the technician in its regular routine. Several repetitions must be carried out by each laboratory so that an estimate can be made of the intra-laboratory reproducibility, later on to be compared with the inter-laboratory reproducibility.

An important step in the certification is a discussion of the obtained results between all participating laboratories. This may give clues to the detection of possible sources of errors or imprecision and to the estimation of the validity of all results. This general discussion can also be very instructive for the individual laboratory, since it may allow the detection of a possible laboratory bias, and in general gives information of the state of the art in the specific analytical field.

An illustration of what can be achieved in a certification campaign in which several laboratories take part with different analytical techniques is shown in Fig. 2. It gives the results obtained by 13 laboratories for arsenic in the fly ash obtained from the combustion of pulverised coal, material which was a candidate reference material for BCR (13). It can be seen that 5 entirely different techniques were involved, although the large majority of the results were obtained by instrumental neutron activation and atomic absorption after hydride generation. Some results had to be discarded for outlying variances (Lab.n° 10 and 11), or



As in Fly Ash

Fig. 2 Arsenic concentrations ($\mu\text{g}\cdot\text{g}^{-1}$) in fly ash as obtained by 13 laboratories taking part in BCR-certification campaign (13).

for straggling mean (lab. no.12) within the sets of results obtained by neutron activation purely on the basis of statistical considerations. For lack of consistency also the sets of results provided by lab. no. 13 were rejected. The sets 8 and 9 although respectively a straggler of mean and of variance were accepted. Certification with 95% confidence limits of less than + 5% was possible with 9 sets of altogether 78 replicates, using 4 entirely different techniques.

Before the final selection of the valid results some generally accepted statistical treatments must be carried out. The Kolmogorov-Smirnov test as reviewed by Lilliefors (goodness of fit of a distribution) (10), the Dixon test and Chauvenet's criterion (outliers) (11), the Cochran and the Barlett tests (homogeneity of the variances of the laboratory means) (12) are the most popular ones.

Certification is however not always based on analyses done by selected laboratories, but by all laboratories willing to cooperate on a free basis to an organized intercomparison study (round robin analysis). This is the case for the IAEA materials. The procedure used for obtaining the results and especially the method of evaluating the obtained results will have an important impact on the credibility associated with the final reference material. As De Goeij et al. (14) state usually the philosophy adhered in evaluating the results assumes a model in which : (a) most of the data belong to a normally distributed population, (b) the arithmetic mean comes close to the true value and (c) only a minor fraction of the data belongs to other populations. These assumptions imply that fully objective, that is mostly statistical, methods can be applied to reject outlying results and to find a good estimate of the true value and a realistic estimate of the confidence limits. It must however be realized that in trace or ultra-trace analyses the first assumption is often not valid, because systematic deviations in one direction may occur in stead of normally distributed ones. In such cases the true value will lie in the upper or more often in the lower part of the range of data reported. It is generally necessary to apply also some subjective, and thus necessarily arbitrary, but scientifically justified criteria during the evaluation of the results. This was also stated by Dyczbinski (15) after a recent study of data from an intercomparison.

This point was illustrated by De Goeij et al. (14) for the Mn, Cu, Cr and Co content of Milk Powder A-11 from IAEA. For Mn, after rejection of 9 values out of 30 the overall mean of $0.377 \mu\text{g.g}^{-1}$ with 95% confidence limits of 0.296 and $0.457 \mu\text{g.g}^{-1}$ was retained as "recommended value with a satisfactory degree of confidence". The authors showed that there is a substantial difference between the average of the 10 accepted AAS values (0.48 ± 0.06) and of the 9 NAA results (0.30 ± 0.04). There is no indication of a normal distribution, but rather contamination during the AAS analysis and spectral interferences during the INAA seem probable. After the elimination of the results probably affected by such errors and including the 2 results obtained by MS, ES and 2 new results obtained by carefully performed AAS and NAA, the authors suggest a value of $0.28 \mu\text{g.g}^{-1}$. Compared to this value the recommended value is 35% higher and its 95% confidence interval does not cover the newly proposed value.

The recent certification of the earlier mentioned simulated air filters Air-3/1 by IAEA was also the result of an intercomparison study (9). Results were obtained from 31 laboratories, which each had received one lot of 6 spiked filters, sandwiched between two unspiked filters in a polyethylene bag. The results were evaluated as described before for IAEA intercomparisons. Outliers are eliminated at a significance level of 0.05 for the set of laboratory averages. Subsequently the overall arithmetic mean is calculated for the so-called "consensus value". Since the deviations of these overall means from the input values (amount spiked) was quite important (mean deviation 10.7%) also the medians and the modes were calculated. The mean deviations from the input values were reduced to 7% for the medians and 7.8% for the modes. It was decided that 13 elements out of 17 could be certified and for 3 other information values were given. As a result of this evaluation procedure these certified values should however be used with care when judging the performances of a laboratory that uses this material as reference material. This point is illustrated with the results for Zn. Thirty laboratories sent results obtained by AAS (17), NAA (7), AES (3) and XRS (3), of which only one was found to be an outlier. While the input value was $152 \mu\text{g}$ per filter, the overall mean was 141.3 with confidence limits of 135 and 147.5, the overall median was 142.7 and the mode value 143.0. The finally certified value is 143 with as 95% confidence limits 132 and 152. Because the filters were spiked carefully with well known standard solutions a deviation of 6% from the input value is very unlikely (9). The graphical presentation of the laboratory means suggests however that there are three populations of results. The first assumption of a normal distribution is thus not fulfilled. A careful analysis performed at our laboratory by INAA yielded a value of 149 ± 3 .

Such intercomparisons yield finally reference materials with certified values or if the agreement of the data is not very good, depending on the degree of confidence involved the specification is presented in various ways such as recommended value, overall mean, grand mean, information value, probable value, etc. The producers often refrain from indicating intervals of accuracy and are sometimes satisfied with the communication of standard deviations of the laboratory means or an estimated uncertainty without any statistic significance. This reflects only the state of the art. Helpful as such reference materials in relatively new fields may be, their exaggerated significance caused by the printed certificate should be reduced in favour of a controllable and provable description of their accuracy.

In a recent paper Staats (16) demonstrates this statement by the careful determination of 50 to 130 ppm P in 8 standard reference steel samples. The values observed were 14 ppm (min 2, max 26) lower than the certified values. The accuracy of the results was achieved and proved by primary calibration with pure standards with a 95% confidence interval of only 1.4 ppm. Such examples demand imperatively the documented application of primary calibration substances when a standard reference material is produced.

AVAILABILITY OF CERTIFIED REFERENCE MATERIALS USEFUL IN TRACE ANALYSIS

Since the demand for reference materials in the field of trace analysis has grown increasingly in the last decade several national and international institutes and organizations have started certification campaigns often leading to the issuing of certified reference materials. No single organization has in fact sufficient resources to meet these demands on a timely basis. Recently Alvarez et al. (17) claimed that NBS had in the USA requests and proposals for more than 250 new SRM's, of which many in the field of trace constituents. They therefore state that greater international cooperation is essential to expedite certification of needed new reference materials, but also to reduce unnecessary duplication of efforts. Table 1 summarizes some of the most important sources of reference materials useful in trace analysis.

TABLE 1 Sources of Reference Materials

Initials	Source
NBS	Office of Standard Reference Materials, National Bureau of Standards, Washington, D.C. 202234, USA
IAEA	International Atomic Energy Agency, Wagramerstrasse 5, P.O.Box 100, A-1400 Vienna, Austria
BCR	Commission of the European Communities, Directorate-General for Science, Research and Development, Community Bureau of Reference, Rue de la Loi 200, B-1049 Brussels
Bowen	Professor Humphrey J.M.Bowen, University of Reading, Reading, United Kingdom
IUPAC	IUPAC, Secretariate, Banc Count Chambers 2/3, Pound Way Cowley Centre, Oxford OX 3YF, United Kingdom
USGS	US Geological Survey, Reston, V.A. 22092, USA
BAS	Bureau of Analysed Samples Ltd., Newham Hall, Newby, Middlesborough, Cleveland, TS8 9EA, England
CRPG	Centre National de la Recherche Scientifique, Centre de Recherches Pétrographiques et Géochimiques de l'Université de Nancy, B.P. 682, Nancy, France
NIM	National Institute for Metallurgy, 1 Yale Road, Johannesburg, South Africa
CSRM	Canadian Standard Reference Material Project, Department of Energy, Mines and Resources, 555 Booth Street, Ottawa 4, Ontario, Canada K1A 0G1
BAM	Bundesanstalt für Materialprüfung, Berlin, Germany
-	All-Union Scientific Research Center of the State Service for Standard Samples, Moscow, USSR
-	National System of Standard Reference Materials Warsaw, Poland
-	The Iron and Steel Institute of Japan, N° 5 Otemachi 1-Chome, Chiyodo-Ku, Tokyo 100, Japan
SASE	Schweizerische Arbeitsgemeinschaft für Stein und Erden Mineralogisch-petrographische Institut, Sahlstrasse 6, Bern, Switzerland

Environmental and energy related materials

Especially the environmental analysis has recently grown remarkably and has become of great importance. In environmental trace analysis one has to determine these trace contents as accurately and precisely as possible because the results are used to :

1. monitor pollution levels and establish normal values ;
2. establish pathways through the environment and develop mathematical models for them ;

3. control the efficiency and effects of certain pollution abatement measurers, and the application of directives ;
4. calculate the pollution load and protect humans, cattle and crops.

Although it is unrealistic to produce a reference material for every conceivable environmental matrix and every potential pollutant at a variety of concentration levels, a major effort has been spent in this field by a number of organizations.

Table 2 lists the majority of the reference materials available in this field, which includes the compartments air, water and soil, but also some potential sources of environmental pollution such as fossil fuels, sludge, fertilizers, etc. Besides the heavy metals of which Hg and Cd are the most important ones, also some organic components and the S-content of energy related materials are of major concern in trace concentrations. It is obvious from this table that much attention has been paid to coal, fuel oil and fly ash. Several of these NBS materials were so successful that they were soon exhausted and had to be replaced by a new reference material (indicated with "a" in table 2). It is also significant that most of the materials which are in preparation and soon will be issued are multi-component reference materials. Besides the certified constituents often information is given on additional constituents.

TABLE 2 Environmental and energy related reference materials

Material	Identification	Constituents certified
Bitum coal	NBS-1632a	As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mn, Na, Ni, Pb, Se, S, Th, U, V, Zn + inf.*
Subbitum coal	NBS-1635	As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, S, Th, U, V, Zn, + inf.*
Coal	BCR-40	trace elements (in preparation)
Subbitum coal	NBS-2682	S
Bitum coals	NBS-2883-5	S
Coal	NBS-1630	Hg, (Se)
Coal	BCR-65	S, ash
Coal fly ash	NBS-1633a	As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Na, Ni, Pb, Se, Si, Sr, Rb, Tl, Th, U, Zn, + inf.*
Coal fly ash	BCR-38	As, Cd, Co, Cu, Fe, Hg, Mn, Na, Pb, Zn + indic.*
Incin. ash	BCR-176	trace elements (in preparation)
Fuel oil	NBS-1634a	Mn, Na, Ni, Pb, S, Se, V, Zn + inf.*
Fuel oils	NBS-1619-24a	S
Reference foils	NBS-1636-8a	Pb
Oil	NBS-1581	polychlorinated biphenyls
Shale oil	NBS-1580	9 polynuclear aromatic hydrocarbons
Urban partic.	NBS-1648	Al, As, Cd, Cr, Cu, Fe, K, Na, Ni, Pb, Se, U, V, Zn + inf.*
Urban dust	NBS-1649	5 polycyclic aromatic hydrocarbons + inf.*
Air filters	IAEA-Air 3/1	As, Cd, Co, Cu, Fe, Mn, Ni, Pb, Se, U, V, Zn + inf.*
Air filters	NBS-2676b	Cd, Pb, Mn, Zn
Air filters	NBS-2673	SO ₄ , NO ₃
Air filters	NBS-2674	Pb
Air filters	NBS-2675	Be
Air filters	NBS-2679	quartz (clay)
Soil	IAEA-soil 5	Al, As, Be, Br, Ce, Co, Cr, Cs, Cu, Dy, Eu, Fe, Ga, Hf, Ho, K, La, Li, Lu, Mn, Na, Nd, Pb, Rb, Sb, Sc, Sm, Ta, Tb, Th, Yb, U, Zn + inf.*
Soils	BCR 141-3	trace elements (in preparation)
River sedim.	NBS-1645	Al, Cd, Cr, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Pb, Tl, Th, U, V, Zn + inf.*
Estuarine sedim.	NBS-1646	Al, As, Ca, Cd, Cr, Co, Cu, Fe, Hg, Mg, Mn, Ni, P, Pb, V, Zn + inf.*
Lake sedim.	IAEA-SL-1	As, Ba, Br, Cd, Ce, Co, Cr, Cs, Cu, Dy, Fe, Hf, La, Mn, Na, Nd, Ni, Pb, Rb, Sb, Sc, Sm, Th, U, Yb, V, Zn, Ti, + inf.*
Marine sedim.	IAEA-SM 1/2	trace elements (in preparation)
Sewage sludges	BCR-144-6	trace elements (in preparation)
Phosph. rock	BCR-32	As, B, Cd, Cr, Co, Cu, Hg, Mn, Ni, Ti, V, Zn + indic.*
Waters	NBS 1643a	Ag, As, Ba, Be, Cd, Cr, Co, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sr, V, Zn
Waters	NBS-1641-2a	Hg
Water	IAEA W 3/2	trace elements (feasability study)
Water	BCR	trace elements (feasability study)
Water	BCR	6 polynuclear aromatic hydrocarbons in isooctane
Water	NBS-1644	generator column for 3 polynuclear aromatic hydrocarbons
Water	NBS-1647	16 polynuclear aromatic hydrocarbons in acetonitrile

* in addition information or indicative values are given

Considerable effort has also been spent to produce solutions of known concentrations of compounds which might be useful in environmental analyses, for the preparation of solutions of a similar composition as those obtained in the analysis or for the application of a type of standard addition procedure by adding a known amount to the matrix to be studied. Examples are SRM-1644 and 1647 (PAH as a generator column or in acetonitrile which is miscible with water) from NBS. Also a variety of organo-metallic compounds are available from NBS (SRM 1051b - 1080a) and from BCR (34-36, 73, 127).

Besides those mentioned in table 2 a large variety of gas mixtures (SO_2 , CO , CO_2 , NO , CH_4 , C_3H_8 and C_6H_6 in air or N_2) and permeation devices (SO_2 , NO_2 , C_6H_6), useful in the analysis of gaseous pollutants is available from NBS. BCR is in the process of preparing similar materials (18). In addition reference materials exist for isotopic ratios and for radioactivity (NBS and IAEA).

Biological and botanical materials

The demand for reliable analytical data for trace elements in human, animal and plant materials continues to increase because the scientific world tries to establish optimum levels for nutrient elements in the diet and to determine the normal as well as the toxic levels of potentially hazardous elements in animal and human tissues. Therefore a variety of botanical and animal reference materials has and is being produced by NBS, IAEA, BCR and IUPAC. A selection is given in table 3.

Several of these materials such as Bowen's kale powder, the NBS bovine liver and the orchard leaves have been of enormous value to the scientific community. But at the same time they have given rise to long lasting controversies and discussions between researchers working in this field. As discussed before the contamination-free preparation and homogenization of a large amount of these materials is a difficult and cumbersome task. Since the concentrations involved are often far below the ppm level also the analyses are very difficult. As a result of this a wealth of information is available in literature on some of these materials. The certificate for the orchard leaves (SRM-1571) gives information on the concentrations of 30 elements, while at least for 10 additional data are to be found in literature. Although this reference material is not available anymore, and is being replaced by the citrus leaves, it is still an important verification material for the labs which have a certain stockpile of it.

Marine animals are of special interest because some of them (bivalves) are known to concentrate several heavy metals from the aquatic environment. This explains the presence of oysters and fish in the list. Obvious is also the abundance of reference materials for cereals, to respond to the demand of food and medical researchers. The large consumption of flour and rice may give rise to accumulation of Hg, Pb and other heavy metals. For similar reasons there is an increased interest in milk powder. Reference materials for trace elements in human tissue, blood, serum or urine are still rare, owing to the enormous problems involved in their preparation. It should be stressed that, partly as a consequence of this, certified values of concentrations in the ppb or sub-ppb level in biological materials are practically non-existing. This is obviously a significant shortcoming.

For toxicology research and monitoring the human exposure in a workplace environment, NBS prepared some sets of freeze-dried human urine (SRM 2670-2672). These sets contain bottles with certified elements at normal and elevated levels. Also the metals, sulfate, nitrate and quartz on filter media (2673-2679) were developed for this purpose.

Primarily to enable the study of the pathways through an entire eco-system of some heavy metals BCR has recently outlined (19) a programme for future work on reference materials needed in the field of environmental and biological field. Reference materials should be made available and thus ameliorate the analytical procedures and capabilities of the specialized laboratories, in the following fields :

- industrial and energy related pollution (coal, fuel, ash, slag, air, airborne dust, rain water, etc.) ;
- water pollution (sewage sludge, fresh water, potable water, river sediment, aquatic plants, fish, etc.) ;
- marine pollution (seawater, marine sediment, plankton, algae, molluscs, fish, etc.) ;
- agricultural pollution (rainwater, fertilizers, pesticides, herbicides, sludges, soils, plants, animal tissues, vegetables, etc.) .

In most of these materials the heavy metals should be studied with emphasis on Hg, Pb, Cd and As, although others such as Cu, Ni, Cr, Zn, V, Se, Mn, Tl, Mo and Sn are also of interest. Since the toxicity of an element heavily depends on its chemical form, the problem of the speciation is more and more put forward. As a matter of fact also a number of organics should be considered for certification (PAH's, organo-chloro pesticides, PCB's, dioxins, etc.). These demands set even more severe requirements to the preparation, stability and analysis of reference materials. The attempt made thusfar to certify the speciation of elements is still in its infancy.

TABLE 3 Botanical and biological reference materials

Material	Identification	Constituents certified (recommended)
<u>Plant-material</u>		
Spinach	NBS-1570	Al, As, Ca, Cr, Cu, Fe, Hg, K, Mn, P, Pb, Rb, Sr, Th, U, Zn + inf.*
Citrus leaves	NBS-1572	Al, As, Ba, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sr, Zn + inf.*
Tomato leaves	NBS-1573	As, Ca, Cr, Cu, Fe, K, Mn, P, Pb, Rb, Sr, Th, U, Zn, + inf.*
Pine needles	NBS-1575	Al, As, Ca, Cr, Cu, Fe, Hg, K, Mn, P, Pb, Rb, Sr, Th, U + inf.*
Brewers yeast	NBS-1569	Cr
Kale powder	Bowen	(Al, As, Ba, Br, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Se, Si, Sn, Sr, Tl, U, V, Zn)
Olive leaves	BCR-CRM 62	Cd, Cu, Hg, Mn, Pb, Zn + indic.*
Aquatic plants	BCR-CRM 60-61	Cd, Cu, Hg, Mn, Pb, Zn + indic.*
Cotton cellulose	IAEA-V 9	trace elements (in preparation)
Wheat flour	NBS-1567	Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Se, Zn + inf.*
Rice flour	NBS-1568	As, Ca, Cd, Co, Cu, Fe, Mg, K, Mn, Na, Se, Zn + inf.*
Wheat flour	IAEA-V 5	I
Wheat flour	IAEA-V 2	Hg
Corn flour	IAEA-V 1	Hg
Wheat flour	IAEA-V 2/1	(As, Co, Cu, Fe, Hg, Mn, Zn)
Rye flour	IAEA-V 8	(Br, Ca, Cl, Cu, Fe, K, Mg, Mn, P, Rb, Zn + inf.*)
Rye flour-normal	BCR	trace elements (in preparation)
Rye flour-grown on sewage sludge treated soil	- BCR	: trace elements (in preparation)
Brown bread lyophil.	BCR	trace elements (in preparation)
<u>Animal-material</u>		
Bovine liver	NBS-1577a	As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, N, Na, Rb, Se, Zn and 7 other + inf.*
Oyster tissue	NBS-1566	Ag, As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, U, Zn + inf.*
Dried oysters	IAEA-MA-M 1	(Cd, Cu, Fe, Hg, Pb, HC + inf.*
Fish meat	IAEA-MA-M 2	(trace elements + HC)
Fish solubles	IAEA-AC	(As, Co, Cr, Cu, Fe, Hg, Mn, Se, Zn)
Meats	BCR	trace elements (in preparation)
Animal blood	IAEA-A 2	(Co, Cu, Fe, Mn, Pb, Se, Zn)
Milk powder	IAEA-A 7	(Ca, K, Na, Sr)
Milk powder	IAEA-A 11	(Ca, Cl, Cu, Fe, Hg, K, Mg, Mn, Na, P, Rb, Zn)
Milk powder normal	BCR-63	Cd, Cu, Hg, Mn, Pb, Zn, nitrate (in preparation)
Spiked skim powder	BCR-150-1	Cd, Cu, Hg, Mn, Pb, Zn, nitrate (in preparation)
Muscular tissue	IAEA-H 4	Br, Ca, Cl, Cs, Cu, Fe, Hg, K, Mg, Mn, Na, Rb, Se, Zn
Animal bone	IAEA-H 5	(Ba, Br, Ca, Cl, Fe, K, Mg, Na, P, Pb, Sr, Zn)
Horse kidney	IAEA-H 8	Cd + trace elements (in preparation)
Lyophil. blood	IAEA-A 13	trace elements (in preparation)
Bovine blood	BCR-194-6	Pb, Cd (in preparation)
<u>Human material</u>		
Human serum	IAEA-HC	I, I bound to proteins
Human serum	NBS-909	Ca, Cl, K, Li, Mg, organ.comp.
Human serum	NBS-900	drugs
Lyophil.human serum	IUPAC	Se (in preparation)
Single cel proteins	BCR	As, Cd, Hg, Pb, Sb, Se (in preparation)
Freeze-dried urine	NBS-2670	As, Ca, Cd, Cl, Cu, K, Mg, Mn, Na, Ni, Pb, Se, Zn
Freeze-dried urine	NBS-2671a	F
Freeze-dried urine	NBS-2671b	Hg

* In addition information or indicative values are given.

Some animal and plant tissues were certified by IAEA for radioactive nuclides (not given in table 3).

Reference materials for traces in other matrices

A large variety of reference materials useful in geological and geochemical analysis exists. Since some excellent compilations of these were made by Flanagan (20) and more recently by Becker et al. (21) no attempt was made to summarize these in the present paper. Very active in the production of such samples have been the US Geological Survey, the National Institute

for metallurgy of South Africa, the Canadian Standard Reference Materials Project, the Centre National de la Recherche Scientifique of France. Also many nations such as the Federal Republic of Germany, the USSR, Poland, Japan and Great Britain have produced reference materials in this field, largely for national use. It is however noteworthy that very few of these materials have been certified. Mostly only recommended or reference values are given.

Special types of material represent the glasses (SRM 608-619) prepared by NBS with data on up to 35 certified elements in concentrations varying from 0.02 to 500 ppm. Because this matrix is rather artificial and the elemental ratios are not similar to those present in any environmental, biological or geochemical material, their applicability in a quality assurance program is not immediately obvious. They have however been used extensively in accuracy tests of newly developed analytical procedures and for the calibration of instruments.

In the field of metals, alloys, ores and minerals the interest in trace and ultra-trace composition has also increased. Therefore a variety of reference materials is available from for example NBS, BCR and a number of national organizations. Interesting in this respect are the ppm and even ppb amounts of light elements such as O, C, N, B which have been certified with relatively low uncertainty limits in non-ferrous metals. Discussion of the existing materials and of those in preparation in this extensive field is out of the scope of this paper.

CONCLUSION

In an increasing number of fields of science and technology there is nowadays an overwhelming demand for accurate data on trace and ultra-trace concentrations of inorganic and organic components. As a result of this the requirements for the analytical laboratory become always more stringent. The analytical data must be valid, accurate and reliable because often widespread and far reaching social, political and economic decisions are made upon these data. It is mostly not by the use of complex sophisticated and automated instrumentation alone that this can be achieved. Errors occurring due to contamination or losses during the handling and pretreatment of the samples, are often the main reasons for inaccuracy or imprecision. Therefore each analytical laboratory must have some type of quality assurance program.

Certified reference materials play a vital role in such a program, since their use is not only complementary to the application of reference methods, but generally also much less demanding and costly. Also participating to intercomparison is a good way to improve the laboratory performances. It has in fact been shown in the past that such a system of frequent interlaboratory matching is of great value to all the participants. The cost of such an organization and participation should however not be underestimated. The obvious solution to this problem, that is avoid the high costs and still be able to detect systematic errors and keep the performances of the laboratory up to date is the use of certified reference materials. Each time a laboratory analyzes a certified reference material, it is as if it takes part in an intercomparison with a number of the best laboratories, with the additional advantage of not having to wait a number of months for the outcome of the intercomparison. Immediately one obtains some information in the state of the art in the laboratory. One must warn however for the comparison with informative or indicative values, overall means, consensus values, etc., because agreement or deviation with such values may guide the laboratories to wrong conclusions. Only the values, certified by an organization with a high credibility, and those with narrow confidence intervals are really useful in a quality assurance program. Moreover they must be used as directed regarding minimum homogeneous quantities and handling procedures. Verification rather than calibration should be the objective. Simple numerical calibration for an observed discrepancy is very dangerous.

Certification campaigns for trace constituents in a variety of matrices are difficult and painstaking care is needed in the selection of the cooperating laboratories and analytical methods. In the selection of the acceptable values statistical methods have of course an important role to play but also analytical considerations on likely interferences, sample pretreatment, selectivity and applicability of the method to the matrix investigated are of utmost importance. When all criteria required for standardization are fulfilled and certification can be achieved all necessary information and helpful data must be provided on the certificate. If even when using the best available method by a choice of excellent laboratories, for certain trace constituents no satisfying agreement can be obtained and only an information or recommended value is given, the laboratory should interpret this simply as a picture of the present state of the art.

In the field of environmental and biological analysis a variety of reference materials has recently been issued or is presently in preparation. The significance of these materials can simply not be overemphasized. Considering the increasing requirement of sensitivity and accuracy the analyst presently has to cope with in a variety of environmental and biological matrices, and for an ever increasing number of trace elements and trace components there is still an urgent need for new better characterized and more accurate standard reference materials. Especially in the biological field certified values at the ppb level are urgently needed. Finally it seems that there is also an increasing demand for microdimensional cha-

racterization. To evaluate the performances of microscopic techniques used in the analytical laboratory such as SEM, TEM, SIMS, LAMA, the microprobe, etc., standardization on the micrometer scale is needed together with some way of certification of the chemical composition of surfaces.

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