USE OF REFERENCE MATERIALS IN THE LABORATORY

What is a reference material? A general definition of a reference material is a material or substance one or more of whose property values are sufficiently homogenous, stable, and well established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials[1]. There are two categories of chemical reference materials used in laboratories, pure substance materials and matrix materials. Matrix reference materials incorporate the analyte in a natural matrix, and have the advantage of identifying matrix effects that may affect the accuracy of the chemical measurement process. There are two broad types of matrix reference materials:

- Certified Reference Material: a reference material issued and certified by an organisation generally accepted to be technically competent
- In-house Reference Material: a material developed by a laboratory for its own internal use

With the large increase in the use of chemicals for the production and manufacture of foodstuffs, pharmaceuticals and consumer products the potential for contamination of air, water, food and the general environment has dramatically increased. Regulation of the level of chemicals in biological and environmental samples is important in trade, community health and pollution prevention. Consequently the trace analysis of both metals and organic contaminants in complex biological and environmental samples is fundamental. There is considerable interest worldwide in the use of matrix reference materials as chemical analysis techniques change from the classical wet chemical techniques to highly sophisticated computer-based comparative test methods.

Reference materials are used for five main purposes in a laboratory:

1. To assist with the development and validation of accurate methods of analysis, ensuring traceable measurement results at a working level.
2. To verify that test methods in current use are performing according to validated performance levels
3. To calibrate measurement systems
4. To assure the long-term adequacy and integrity of measurement quality assurance programs
5. To use as test materials for inter-laboratory comparisons and proficiency test programs

Understanding Reference Material Certificate Information

CRM certificates provide considerable information about the particular standard. Compositional values with uncertainty limits are given for all certified values; these limits are normally 95% confidence intervals. Many reference material suppliers include analyte values for “informational purposes” only, normally these values are not certified because:

- Value determined by only one technique
- Sample homogeneity problems
- Discrepancies between different analytical techniques

Certificates often describe restrictions on the use of the reference material, these must be observed for reliable analytical results. These restrictions include:

- Drying – when drying is critical the certificate instructions must be observed. Some constituents can be determined on a dried standard portion while other constituents which may be volatile are determined with subsequent correction to a dry weight basis.
- Homogeneity – some standards specify a minimum weight due to the heterogeneous nature of the material.
- Segregation – some standards exhibit segregation problems on standing or during transportation. Certificates may instruct the purchaser to reconstitute the standard by mixing. If users fail to follow these instructions they compromise all sample portions taken from the standard container.
- Storage – some standards may require “special” storage conditions, for example refrigeration, freezing or in some cases standards are certified for first only use.
- Shelf life – some standards may have a finite life and certification is only valid for that lifetime.

Certificate instructions must be observed to maintain sample integrity and to ensure results consistent with certified values.

Using Reference Materials

Method Development, Validation and Evaluation

When a new method is developed or an existing method is modified to meet special needs it must be validated. The objective of the process is to identify the performance of the analytical method and to demonstrate that the analytical system is operating in a state of statistical control. Validation procedures are a three-part process:

1. *Determination of key performance characteristics*
   - Limit of detection
   - Accuracy, precision, (bias)
   - Sensitivity
   - Range
   - Selectivity
2. *Analysis of independent unknown samples*
   During this stage of the validation process, reference materials can be used and make the process very simple if the test samples and the reference material have a similar macrocomposition. Reference materials must be fit for purpose, ideally certified reference materials that are matrix matched. The analytical chemist must analyse a sufficient number of reference materials over the method concentration range and compare the results to the expected or certified values. An analytical procedure should be capable of producing results that do not differ from the certified value more than can be accounted for by within laboratory statistical fluctuations. When a suitable reference material is not available, the laboratory may use several other approaches. Spiked samples and the use of surrogates are approaches available to laboratories, these are less desirable than using reference materials because of the difficulty in preparation and because artificially added materials like spikes and surrogates may exhibit different matrix effects from naturally occurring analytes.
3. *Determination of method ruggedness*
   This is a measure of stability of test results produced when steps or operational parameters are varied. Such parameters include the environmental factors of temperature, pressure and relative humidity, and chemical factors such as concentrations of reagents, pH control. By establishing the relative effects of these parameters, one can estimate tolerances within which these parameters must be maintained in order to obtain results within acceptable limits.

Once the new method has been developed and validated, reference materials can be used by laboratories to gain experience and proficiency in its application. The use of certified reference materials eliminates questions of stability and homogeneity that may otherwise complicate interpretation of analytical results from less well characterised samples. Laboratories can also use reference materials to confirm an analyst's ability to use a new method, before undertaking analysis of client's samples.

Direct Calibration of Methods and Instrumentation

Reference materials are used as calibrating standards by a number of different measurement techniques. The National Institute of Standards and Technology (NIST) provide CRM's that define the practical pH scale as well as CRM's for fixed temperature points. When analysing ores and metals X-ray fluorescence (XRF) spectrometry technique is commonly calibrated using reference materials, for example the ASTM Standard Method E322 for low alloy steels recommends 1220 and 1700 series low-alloy steel CRM's as calibrants. Potts[2] refers to the use of reference materials as XRF calibrants for silicate analysis by stating that, it is general practice to calibrate spectrometers against as many reference materials as possible, in part to minimise the effects of errors arising from discrepancies in standard values. The precision of the XRF technique is normally very high while accuracy, on the other hand depends heavily on two factors: the attenuation-enhancement correction procedure used to correct matrix effects caused by concomitant elements, and calibration procedures. Analysis of metals using emission spectrometry also use reference materials for calibration, these are often a combination of both certified metal alloys as well as standards developed in-house.

Measurement Quality Assurance Programs

One of the major uses of reference materials in laboratories is for the quality assurance of analytical measurements. This was in fact the original driving force behind the development of Certified Reference Materials. When unacceptable discrepancies arise in sample results between analysts using different methodologies, or even the same method, the use of reference materials can help identify and resolve the analytical problems. The complexity of modern analytical techniques provides many sources of error, and opportunities for the introduction of bias and imprecision. If analytical results are to be meaningful a laboratory must operate under a rigid quality assurance program. Quality assurance is the name given to the procedures used to ascertain that analytical results are good enough for their intended purpose. Quality assurance involves two distinct but related activities, quality control and quality assessment[3]. A well-implemented quality assurance program will monitor the analytical process for statistical control and alert users when corrective action is required. To this end control charts can be used as simple graphical means of interpreting test data.

Control charts were first developed in 1934 by Dr Walter Shewhart[4] to monitor the outputs of manufacturing processes. In analytical chemistry, control charts are the simplest and most convenient method to monitor accuracy and precision of analytical methods. A control chart can be maintained for any individual repetitive quality control check such as analysis of reference materials, analysis of a constant concentration matrix spike or a matrix spike duplicate, these measurement results are plotted sequentially (time-ordered). X control charts assume that the distribution of values around the mean is binomial and the following distribution should be obtained, Figure 1:

Mean ± 1σ = 68% of observations
Mean ± 2σ = 95% of observations (Warning Limit)
Mean ± 3σ = 99.7% of observations (Control Limit)

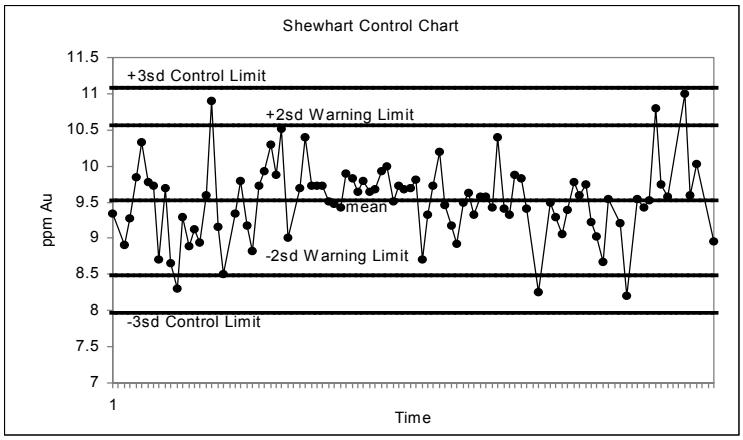

Figure 1. Shewhart control chart showing warning and control limits

Control limits can be based on established limits or experimentally established ones. With reference materials, the certified value of the standard usually serves as the centre line with either established limits or experimentally estimated standard deviation to establish the control and warning limits. Laboratories should not necessarily use the certified value as the centre line if the certificate values represent total content and the test method used by the laboratory only achieves partial extraction. The laboratory must properly validate a recommended value for the partial extraction. This is particular relevant for environmental laboratories undertaking USEPA digests for metals analysis.

It is expected that 5% of quality control results will fall between the warning and control limits. When a sample point falls outside the control limits, the laboratory has reason to believe that the analytical process may no longer be in statistical control. Additionally, laboratories should use control charts to examine systematic trends, for example the existence of runs, that is a series of sample points in the same direction (up or down) or points residing on the same side of the centre line, even though all are within control limits. The term *special* or *assignable* causes as opposed to *chance* or *common* causes was used by Shewhart to distinguish between a

process that is in control, with variations due to random (chance) causes only, from a process that is out of control, with variations that is due to some non-chance factors. The probability of any sample point in an X control chart falling above the centre line is equal to 0.5, provided the system is in statistical control. The probability that two consecutive sample points will fall above the centre line is equal to 0.5 times 0.5 = 0.25. Accordingly, the probability that 9 consecutive points on one side of the mean is equal to 0.5^9 = 0.00195, which is approximately the probability that a sample point can be expected to fall outside the 3σ limit. To use control charts for trend analysis they must be maintained and interpreted on a real-time basis, far too often laboratories prepare charts after a considerable time delay so they only provide a history of analytical performance with little opportunity for the laboratory to control the measurement process. Figure 2, below shows a Shewhart control chart derived from analysis of a CRM over an eighteen-month period, although the laboratory recorded results in a QC data base, they were never used to monitor analytical performance.

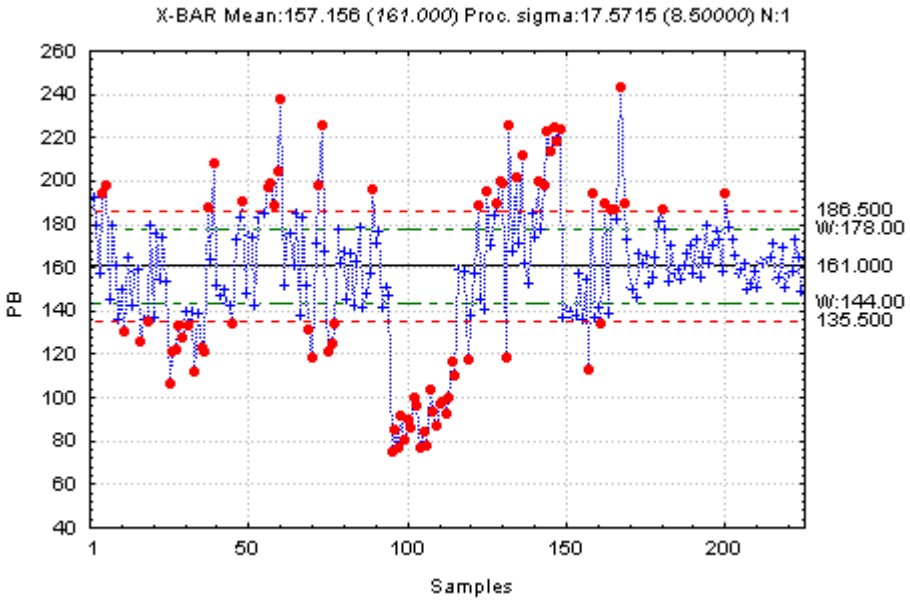

Figure 2. Shewhart control chart for lead analysis of a CRM

Submission of control standards to monitor performance of a laboratory, is a vitally integral part of any exploration or mining project. Control standards monitor accuracy and these standards require inter-laboratory test work so that a “true or accepted” value can be established. Companies should examine the spread of published assay results used to determine recommended standard values on typical industry standards. For a 50g fire assay for gold, after outlier results have been removed, the spread of analytical results averages about 20-30%, while base metal result spread can be anything up to an order of magnitude, see Figure 3. If this is a typical spread for “round robin” sample results, when it can be assumed that laboratories take a little bit of extra care.

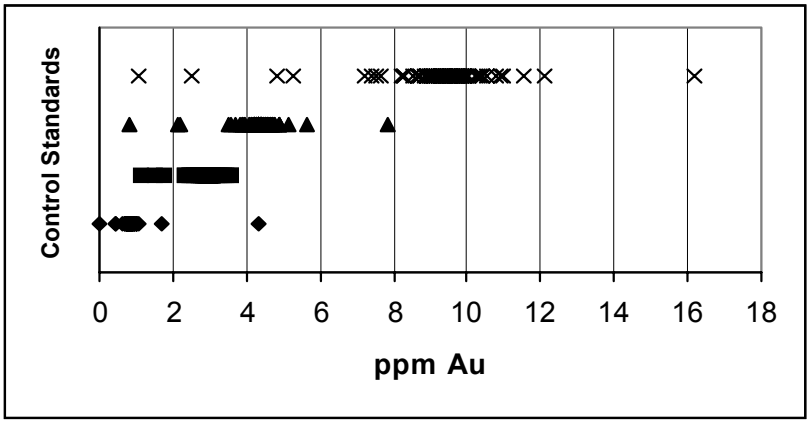

Figure 3. Typical spread of gold assay results obtained in round robin surveys to establish recommended values on four control standards. Data was obtained using 50g fire assay deteminations and includes all statistical outliers.

The mining industry should understand that "not all standards are created equal" and should critically examine the quality and usefulness of available standards. Following this critical assessment, when appropriate control standards have been purchased or developed and validated "in-house", then the identity and concentration of analytes should not be known to the laboratory. It is important that the development of control standards is carefully planned. For large scale projects, reject material can be used and two standards that have similar analyte concentrations for each grade should be prepared, so that laboratories can't differentiate without accurately analysing them[5].

Proficiency Testing Programs

Reference materials are often used in proficiency testing or round robin programs to evaluate the performance of laboratories. If laboratories have a quality assurance program in place that demonstrates that measurement processes are precise and in statistical control, then proficiency testing programs are a useful tool for confirming laboratory precision and identifying measurement bias. If participating laboratories don't possess these prerequisites, then because of the small number of participants in these programs only gross problems can be identified. These programs are to evaluate competency, not to gain it.

Development of In-house Standards

Reference materials, which are produced by laboratories in-house, are generally used on a day-to-day basis. The attraction of using in-house reference materials is that they provide a relatively cheap option as compared to using certified reference materials and can closely resemble the laboratory's routine test samples. Laboratories must remember that in-house reference materials do not replace CRM's, but merely enable CRM's to be used on a less frequent basis. It is vitally important that in-house standards are appropriately prepared to ensure that they are fit for purpose. The following points must be considered when they are produced:

1. *Material selection*

The reference material needs to have the same matrix as the test samples that are being analysed by the method that the in-house standard is intended to control. The following issues need to be addressed:

- Matrix of interest
- Analytes of interest
- Analyte concentration levels
- Degree of homogeneity required
- Stability or shelf life required
- Quantity of reference material required
- Ease of sourcing and sampling
- Health and safety implications

2. *Material Preparation*

The material must be prepared and processed in such a way as to ensure that its final form is fit for intended use and is sufficiently stable. Processes involved in the preparation may include drying, sieving, pulverising, mixing, splitting, filtering, sterilisation etc. During the processing stage care must be taken to prevent change or degradation in species and/or concentration of analytes. The choice of storage container, its closure and internal atmosphere all need to be considered if the integrity of the reference material is to be maintained.

3. *Homogeneity testing*

It is essential that an in-house reference material is homogenous, that is the difference between representative sample measurements must be smaller than the overall uncertainty limits of the measurements. For inorganic constituents this can often be conveniently checked using instrumental neutron activation analysis INAA. INAA is a relatively simple, sensitive, precise and non-destructive analytical technique and is remarkably free from analytical interferences with matrix effects being small, a technique that requires no sample preparation. When evaluating homogeneity each analyte to be given a recommended value must be individually considered, it is not justifiable to examine one analyte and draw a conclusion about the homogeneity of another. Homogeneity must also be coupled with sample size; that is the mass.

Once the in-house reference material has been prepared and tested for homogeneity, recommended values must be established. CRM's can be used to develop secondary reference materials. Several CRM's must be analysed at the same time as the in-house reference material, these CRM's should span the concentration range of interest for each analyte. When sufficient results are obtained the data must be statistically evaluated in order to establish recommended values. Alternatively, the in-house reference material can be sent for analysis to other laboratories, results determined in-house and those obtained at independent laboratories must then be statistically analysed in order to establish recommended values.

Conclusions

Reference materials are fundamental to a laboratory's quality assurance program, allowing the laboratory to verify the accuracy of measurements in a system known to be in statistical control. Besides being useful in the development and validation of laboratory methods, reference materials can be used for the evaluating methodology and for evaluating the proficiency of analytical chemists and laboratories.

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