SIMPLE GUIDE TO QUALITY ASSURANCE

The elements that should be addressed in a quality assurance program include:
- Chain-Of-Custody procedures & sample tracking
- Sample handling & storage procedures including; holding times & preservation
- **Method validation**
- **Method verification**
- Instrument calibration
- **QC samples & standards procedures including; frequency and performance evaluation**
  - Field blank
  - Sample blank
  - Spike Recovery (accuracy)
  - Surrogates (accuracy)
  - Certified Reference Materials (CRM) & in-house standards (accuracy)
  - Field duplicates – (precision)
  - Sample replicates – (precision)
- Internal audits
- Personnel training & qualification
- Equipment capabilities & maintenance program

The elements above that are highlighted in bold will be dealt with in this guide.

Method Validation

When a new method is developed, an existing method is modified to meet special needs or a standard method used outside their intended scope, it must first be validated. The objective of this validation 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.

The following key performance characteristics are to be determined in the validation process:
- Detection limit
- Bias (Accuracy) – the systematic error of the method or closeness of the measured value to the true value for the sample – two approaches could be used:
- Analysis of certified reference materials (CRM)
- Recovery of known amounts of analyte spiked into sample matrix
- Precision – random error introduced by using the method
- Linearity – linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration
- Concentration range – concentration interval over which acceptable accuracy, linearity, and precision are obtained
- Method ruggedness – stability of the result produced when steps in the method are varied
- Matrix effects
- Specificity

Detection Limit

A measured value becomes believable when it is larger than the uncertainty associated with it. The point at which this occurs is called the detection limit. The Method Detection Limit (MDL), which takes into account sample preparation and analytical conditions, as approximately 2.3 times the standard deviation of analytical results, at a concentration of between 1 and 5 times the expected MDL determined by at least seven (7) replicate measurements.

$$\text{Calculated by} \quad MDL = t \times s$$

Where t = taken from a one-sided t distribution for n-1 degrees of freedom
         s = standard deviation of analytical result

The lower level where measurements become quantitatively meaningful is called the Practical Quantitation Limit (PQL) and is arbitrarily defined as five (5) times the MDL.

Bias

Bias is the systematic error inherent in a method or caused by an idiosyncrasy of the measuring system (e.g., blanks, contamination, mechanical loss and calibration errors). Bias may be both positive and negative, and several kinds can exist concurrently so that net bias is all that can be evaluated. The bias of an analytical method is usually determined by study of relevant reference materials or by spiking studies.

Bias is established by analysing ten (10) replicate aliquots of sample matrices spiked at two concentrations. Spiking levels are five (5) times the PQL and one relatively high so that the range of concentrations for which the methods is applicable is specified, i.e. 50 times the PQL. Method bias (accuracy) is determined at both concentrations by calculating the mean recovery of the spiked analytes for the ten (10) replicates.

$$\text{Bias} \quad = \quad \left(\frac{\bar{x}}{X} \right) \ x \ \ 100 \ \%$$

where
- $\bar{x}$ = Mean value for the ten (10) replicates
- X = Spiked concentration

Precision

Method precision is determined by calculating the percent relative standard deviation (%RSD) of the spiked analyte recoveries for the ten (10) replicates at each concentration.

$$\text{Precision} \quad = \left(\frac{s}{\bar{x}} \right) \ \ x \ \ 100 \ \%$$

where:
- $\bar{x}$ = Mean value for the ten (10) replicates
- s = standard deviation for the ten (10) replicates

Specificity / Matrix Effects

Though loosely defined relates to the degree to which a method responds uniquely to the required analyte. Typical selectivity studies investigate the effects of likely interferents, usually by adding the potential interferent to both blank and fortified samples and observing the response. The results are normally used to demonstrate that the practical effects are not significant.

Method Ruggedness

Method ruggedness is a measure of stability of test results produced when steps or operational parameters are varied. Parameters that can be varied include the environmental factors of temperature, pressure and relative humidity, and chemical factors such as concentrations of reagents, pH control, etc. When the laboratory establishes the relative effects of these parameters, we can estimate tolerances within which these parameters must be maintained in order to obtain results within acceptable limits.

Method Verification

When a laboratory uses standard methods, then methods must be verified to ensure that the laboratory's analytical performance is as specified in the standard method, i.e. APHA methods etc. Verification methodology involves the determination of:

- Detection limit
- Bias (Accuracy) – the systematic error of the method or closeness of the measured value to the true value for the sample – two approaches could be used:
- Analysis of certified reference materials (CRM)
- Recovery of known amounts of analyte spiked into sample matrix
- Precision – random error introduced by using the method
- Linearity – linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration
- Concentration range – concentration interval over which acceptable accuracy, linearity, and precision are obtained

QC Samples & Standards

Glossary of Terms

1. **Batch** Samples being prepared or analysed together
2. **CRM** Certified Reference Material - a reference material issued and certified by an organisation generally accepted to be technically competent
3. **MDL** Method Detection Limit
4. **PQL** Practical Quantitation Limit
5. **RPD** Relative Percent Difference

Control Charts

Control charts are the simplest and most convenient method to monitor accuracy and precision of test 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 with the following distribution:

Mean ± 1σ = 68% of observations

Mean ± 2σ = 95% of observations (Warning Limit)

Mean ± 3σ = 99.7% of observations (Control Limit)

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, a laboratory 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 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.
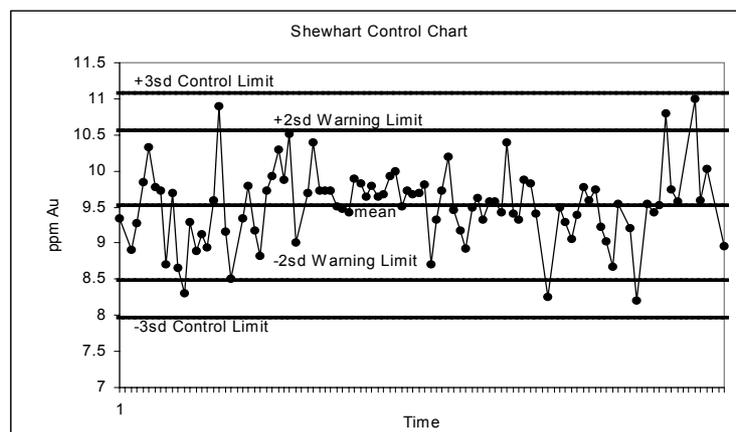
Field Blank

When Laboratory Quality Management Services P/L undertakes the sampling responsibilities for a customer project or contract, sampling personnel include a field blank with all sample batches. High-purity water is placed in an appropriately prepared sample bottle at the sampling location in the field. If the samples are filtered in the field then the high-purity water will also be filtered, preservative is add if appropriate and the sample submitted with other samples collected during the sampling operation. The field blank serves to check the purity of preservatives and laboratory water used during the sampling operation; it also gives an indication of the sample contamination picked up at the sampling site.

Sample Blank

Sample blanks are the first analysis performed in any sample batch to determine the level of contamination in reagents, glassware, and preparative steps etc. The sample blank results must fall within agreed acceptance criteria before sample analysis is allowed to continue. The degree of control of the analytical blank, i.e., contamination from all sources external to the sample, seriously affects the accuracy of low-level trace determinations. To improve and lower detection limit of trace determinations it is imperative to control the variability of the analytical blank results arising from contamination arising from four principal sources:

- Laboratory environment in which the analysis is performed
- Reagents used in the analysis
- Apparatus used
- Analyst performing analysis

The laboratory should include one (1) sample blank every twenty (20) samples or batch if smaller. The acceptance criteria for the sample blank should be twice the practical quantitation limit (PQL), if the sample blank fails to meet the acceptance criteria, a blank and 10% of the sample batch and new QC samples should be analysed.

Spike Recovery

A matrix spike refers to the addition of a known amount of analyte to a sample. The analysis of the sample and the matrix-spiked generate an analyte recovery and this recovery provides the laboratory an indication of the bias of the test method for that environmental sample matrix. This bias can be either positive or negative and causes recoveries greater than or less than 100%. Comparison of the matrix spike recoveries with blank spike accuracy is used to assess whether the analytical process is in statistical control. Poor performance of the test method in a number of different sample matrices indicates the method might not be in statistical control, even if blank spike accuracy is acceptable. For example, interferences from environmental samples might cause bias in the recovery of matrix spike analytes, indicating corrective action might be needed.

Matrix spike duplicates can be used to calculate test method precision. Matrix spike duplicates are used to estimate method precision for analytes that are frequently found below the PQL. A second sample aliquot (same as matrix spike) is taken through the analytical process. The relative percent difference of the matrix spike and the matrix spike duplicate is calculated and used to assess analytical precision.

$$\% \text{ Recovery} = \left(\frac{C_{spiked} - C_{unspiked}}{C_{actual}} \right) \; x \; 100$$

Where:

C_{spiked} = measured concentration in spiked sample

$C_{unspiked}$ = measured concentration in unspiked sample

C_{actual} = actual concentration of spike added

$$\text{RPD} = \left(\frac{C_{higher} - C_{lower}}{\bar{x}} \right) \; x \; 100$$

Where:

C_{higher} = concentration of the higher of the two observations

C_{lower} = concentration of the lower of the two observations

$\bar{x}$ = mean concentration of the two observations

Matrix spike and matrix spike duplicates should be run every twenty (20) samples or job if smaller. Percent recovery should generally fall within the pre-determined acceptance limits.

Surrogates

Surrogates are organic compounds, which are not found in environmental samples, but have a similar chemical structures, and extraction and/or chromatography properties. Surrogates are spiked into ALL blanks, calibration & QC standards and samples, including replicates, prior to analysis by GC or GC-MS. The surrogates are calibrated just like requested analytes and are used to evaluate the sample preparation process. Since surrogates are used to highlight sample preparation problems they must be very similar to requested analytes.

Surrogate compounds and their acceptable recovery ranges should be specified in test methods. Recovery data is reported with all sample results. When a recovery value does not fall within acceptance limits, calculations and surrogate solutions are rechecked. If samples and/or extracts are checked are still not within acceptance limits, a note will be included on the test report.

$$\% \ Recovery \quad = \quad \left(\frac{C_{measured}}{C_{added}} \right) \quad x \quad 100$$

Where:

$$C_{measured} \quad = \quad \text{measured concentration of surrogate}$$

$$C_{added} \quad = \quad \text{actual concentration of surrogate added}$$

Reference Materials

Reference materials are fundamental to a laboratory's quality assurance program; it allows them to verify the accuracy of measurements in a system known to be in statistical control. With reference materials, the certified or established 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.

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

Reference materials should be run every twenty (20) samples or job if smaller. All reference material data should be plotted on control charts and examined in real time against acceptance criteria. Control charts must also be examined for trend analysis.

Certified Reference Materials

Certified reference materials are used for five main purposes in the laboratory:
- To assist with the development and validation of accurate test methods
- To verify that test methods in current use are performing according to validated performance levels
- To calibrate measurement systems
- To assure the long-term adequacy and integrity of measurement quality assurance programs
- To use as test materials for inter-laboratory comparisons and proficiency test programs

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:
- 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 that 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.

In-house Reference Materials

Reference materials, which are produced by laboratories in-house, are generally used on a day-to-day basis. The attraction of using in-house laboratories is that they provide a relatively cheap option as compared to using certified reference materials and closely resemble our customer's routine test samples. It must be emphasised that in-house reference materials do not replace the use of CRM's, but merely enable us to use CRM's 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 are always considered when we produce in-house standards:

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
- 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

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.

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.

Establishing Recommended Values

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.

Field Duplicates

When Laboratory Quality Management Services P/L undertakes the sampling responsibilities for a customer project or contract, sampling personnel must include field duplicates with all sample batches. Field duplicates are two separate samples collected at the same time and sampling location under identical conditions and then treated exactly the same throughout the laboratory processes. The results obtained for field duplicates give a measure of the precision associated with sample collection, preservation, storage as well as the test methods. Field duplicates are normally taken on 5% of sample volume, but this may vary with individual customer contracts. Field samples should not be confused with sample splits.

Sample Replicates

Two sample portions are taken from the same sample container then processed and analysed as two separate samples. Analysis results are used to measure analytical precision from the sample digestion/extraction step through the analysis process.

Laboratories should include one (1) sample replicate every twenty (20) samples or batch if smaller. The laboratory should have pre-determined acceptance criteria for sample replicate results, i.e., within 15% RPD of the mean two results. If sample replicates fail to meet the acceptance criteria, the laboratory should repeat 10% of the sample batch including new QC samples and blank. If replicate results are within acceptance criteria both results are reported on test reports. Acceptance criteria do not normally apply at concentrations less than five (5) times PQL.

$$\text{RPD} \quad = \quad \left(\frac{C_{higher} - C_{lower}}{\bar{x}} \right) \quad x \quad 100$$

Where:

C_{higher}	=	concentration of the higher of the two replicates
C_{lower}	=	concentration of the lower of the two replicates
$\bar{x}$	=	mean concentration of the replicates

Sample Splits

Sample splits are aliquots of samples taken after the sample preparation process i.e. pulverising, mixing or sample compositing. The samples are independently analysed and are often used to evaluate intra- or interlaboratory precision. Customers often send sample splits to a second laboratory to evaluate their primary laboratory's analytical performance