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Chapter 4*: QUALITY ASSURANCE

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Quality Assurance (QA) is a management method that is defined as “all those planned and systematic actions needed to provide adequate confidence that a product, service or result will satisfy given requirements for quality and be fit for use”. A Quality Assurance programme is defined as “the sum total of the activities aimed at achieving that required standard” (ISO, 1994).

Any monitoring programme or assessment must aim to produce information that is accurate, reliable and adequate for the intended purpose. This means that a clear idea of the type and specifications of the information sought must be known before the project starts, i.e. there must be a data quality objective. Data quality objectives are qualitative and quantitative specifications that are used to design the system that will limit the uncertainty to an acceptable level within the constraints allowed. These objectives are often set by the end users of the data (usually those funding the project) in conjunction with the technical experts concerned.

Quality Assurance for a recreational water monitoring programme will, apart from helping to ensure that the results obtained are correct, increase the confidence of funding bodies and the public. Quality Assurance extends to all aspects of data collection from sanitary surveys to laboratory procedures. Unless the data can be checked they should not be included in any assessment; unconfirmed observations have little value and can result in misclassification.

4.1 Components of Quality Assurance

The components of a QA programme are often grouped into three levels, variously labelled: the strategic or organisational level (dealing with the quality policy, objectives and management and usually produced as the Quality Manual); the tactical or functional level (dealing with general practices such as training, facilities, operation of QA); and the operational level (dealing with the Standard Operating Procedures (SOPs) worksheets and other aspects of day to day operations).

4.1.1 Setting up the system

There is no single method for establishing a QA system. Each organisation has its own problems that will require special consideration and planning. However, once the decision to implement a QA system has been taken and the necessary funds and facilities have been made available, then a plan must be drawn up. For a new project the QA system can be drawn up before the start but if the project is already established then a QA system can be retrofitted. In the latter situation, existing practices must be evaluated with respect to QA needs and any QA checks and procedures that are already in place. It is better to build on procedures already in place and only to remove them if they are clearly unsatisfactory. If too many changes are imposed too quickly, especially where they are seen to increase work load, they are unlikely to be met with a favourable response and implementation will be poor. The QA programme must be seen to be practical and realistic and not to include trivial or unnecessarily time-consuming or difficult tasks (WHO/UNEP/VKI, 1997).

4.1.2 The Quality Manual

The Quality Manual is composed of the management documents needed to implement the QA programme and includes (ISO, 1990):

- A quality policy statement, including objectives and commitments.

- The organisation and management structure of the project, its place in any parent organisation and relevant organisational charts.

- The relationship between management, technical operations, support services and the quality system.

- Procedures for control and maintenance of documentation.

- Job descriptions for key staff and reference to the job descriptions of other staff.

- Identification of approved signatories.

- Procedures for ensuring traceability of all paperwork, data and reports.

- The laboratory's scope for calibrations and tests.

- Arrangements for ensuring that all new projects are reviewed to ensure that there are adequate resources to manage them properly.

- Reference to the calibration, verification and testing procedures used.

- Procedures for handling calibration and test items.

- Reference to the major equipment and reference measurement standards used.

- Reference to procedures for calibration, verification and maintenance of equipment.

- Reference to verification practices including inter-laboratory comparisons, proficiency testing programmes, use of reference materials and internal quality control schemes.

- Procedures to be followed for feedback and corrective actions whenever testing discrepancies or departure from documented procedures are detected.

- Procedures to be followed for feedback and corrective actions whenever testing discrepancies or departure from documented procedures are detected.

- Complaints procedure.

- Procedures for protecting confidentiality and property rights.

- Procedures for audit and review.

4.1.3 Training

The development of the programme must include all staff. Typically, the management commit resources, establish policy and standards, approve plans, assign responsibilities and maintain accountability. The supervisory staff take responsibility for the development and implementation of the programme and operating personnel provide technical expertise and advice. At all stages, the operating personnel must be consulted about the practicalities of any proposed changes. In turn, they must notify management of any problems or changes that may affect the programme.

4.1.4 Standard Operating Procedures

Standard Operating Procedures (SOPs) are the documents detailing all specific operations and methods, including sampling, transportation, analysis, use of and calibration of equipment, production of reports and interpretation of data. They are the internal reference manual for the particular procedure and should detail every relevant step. Anybody of the appropriate training level should be able to follow the SOP. They should, where necessary, cross-reference other SOPs and refer to them by number. Method SOPs may originate from organisations such as the International Organization for Standardization (ISO), British Standards Institute (BSI), American Standard Technical Method (ASTM) or from the instructions that come with the test kit where a commercially produced method is used. Such SOPs have the advantage of not requiring verification and save time in writing “in-house” SOPs. However, if they are used they must be used without modification. If any modification at all takes place, the alterations must be documented. Sometimes “in house” methods are preferred, and it is vital that such methods are properly verified. This may be done by reference to scientific literature and by “in house” validation.

The procedure should be written in short, clear sentences. Equipment SOPs should include methods and frequency of maintenance, cleaning, calibration and servicing. Method SOPs should include all the information necessary to carry out the procedure without reference to other documents with the exception of fully documented SOPs. Any

statements regarding ranges for measurement variables such as temperature, weights, etc. should be within the scope of the facility, i.e. not so wide that they affect the result but not so narrow that they are not practically achievable or necessary. Calculations should include any equations and demonstration of statistical control. Where applicable, criteria for the acceptance of data should be stated and acceptable ranges quoted. Disposal methods for reagents, test materials and other consumables should also be stated.

Some SOPs, such as those for office procedures, will be customised. The person most technically competent to carry out the procedure described should write the SOP. An SOP should have a descriptive title and also have a unique reference and version number. The purpose of the SOP should be stated alongside the variables measured, the expected range of values, the limitations of the method and the expected precision and accuracy. Any documents regarding the source of the method should be stated. Safety notes should include any foreseeable risks involved in the procedure, alongside procedures to minimise risk and procedures in case of an accident. Any special training required for the operator, and special apparatus required for the procedure (including all reagents and materials required) should be stated along with such information as the grade, reference number, size and company of origin. The storage, handling, recording and subsequent disposal of the sample should also be covered in the SOP, including storage temperatures, sample splitting, traceability, and any other issues. The style and format of the final data report should be given where applicable and reporting procedures and archiving requirements should also be included.

4.1.5 The Quality Assurance manager

For larger projects, proper management of QA will require the appointment of a QA manager to liaise with staff, to manage data archives, to conduct regular audits and reviews and to report on any QA issues. The manager is responsible for inspecting all aspects of the system regularly to ensure compliance, for reporting on such inspections and audits to management and for recommending improvements. These activities involve inspecting facilities and procedures regularly, tracing samples and documents back through the system and ensuring that all appropriate records have been kept.

Where QA is the responsibility of a separate section within an organisation many of the management difficulties are minimised. Appointment of a full time QA manager is difficult in a small organisation and in these cases the responsibility for QA should be assigned on a part-time basis, to a suitable member of staff.

4.1.6 Auditing and checking compliance

When all the documentation for the QA system is in place, it should be piloted. During this time, the QA manager should conduct a series of audits covering all aspects of the system. Traceability of data is a key component which can be checked by picking data at random and tracing them back through all relevant paperwork to the sampling procedure. A review of the system with positive and negative areas clearly defined should be written at the end of the pilot phase.

One method of implementation is to apply for accreditation from a recognised QA system. The ISO standard, ISO 9000, is suitable for the monitoring programme as a

whole and is available in many countries. These systems are expensive but do allow the QA programme to be assessed independently against an agreed standard. Sometimes formal accreditation is required by regulatory and commercial bodies.

4.1.7 Maintaining Quality Assurance

In order to maintain the QA system, it is necessary to check periodically each area of the system for compliance. This involves auditing the component parts to assess whether they continue to meet the original criteria. This procedure should be formerly documented. Reports on all audits should be made available to management and to the persons responsible for the work concerned. Deviations from required standards must be corrected as soon as possible. The audit must be independent, and should be thorough and unannounced.

4.2 Equipment maintenance and calibration

All equipment, whether site, office or laboratory, must be maintained on a regular basis as documented in the relevant SOPs, codes of practice and manufacturer's guidelines. Laboratories must apply standards within the limits established for the care of a particular piece of equipment. This applies to general equipment, such as glassware, as well as to sophisticated analytical instruments and vehicles. It especially applies to field equipment.

The care and cleaning of equipment is very important to ensure analytical quality. Regular internal and external calibration checks must be performed on equipment such as balances, pipettes and pH meters. The frequency of these checks depends on the stability of the equipment in question but should be based on established practice. The form and frequency of these checks should be documented in the relevant SOPs. Calibration and maintenance records should be kept for all equipment, thus allowing the repair status to be monitored.

4.3 Sampling

Any analysis can only be as good as the sample taken. Variations in sampling procedures can have a marked effect on the results of analysis. It is very difficult to quantify these effects and therefore procedures for sampling operations should be documented carefully so that all relevant information is recorded at the time of sampling by the field worker.

4.3.1 The sampling plan

For any sampling programme, a sampling plan must be prepared to allow full control of the sampling process so that any change seen between two sampling rounds can be attributed to changes in environmental conditions and not to changes in procedure. Items to be considered in preparing a sampling plan include planning issues, fieldwork procedures and field safety issues.

Planning issues

Planning issues include identification of the objective of sampling (e.g. to test compliance with a bathing water regulation), choice of site (location, type of water body), the type and number of samples to be collected (sample types, e.g. water, sediment, the number of samples, appropriate equipment) and timing of sampling (considering the state of tides).

Fieldwork procedures

Consideration must be given to sampling SOPs (for equipment, sampling method, storage, etc.), as well as size of sample and sample containers. Preservation must be decided in consultation with staff from the analysing laboratory, who will advise clients on the volume and type of sample and who will usually provide sampling containers and preservatives where necessary. Ensuring field quality control includes the use of blanks, duplicate samples, replicate samples and spiked samples. Storage and holding time (conditions for storage, such as in an ice box, maximum time before analysis for unstable parameters, etc.) must also be considered.

The laboratory staff must be made aware when samples are due to arrive so that they can make the appropriate arrangements. When choosing an analytical laboratory it is important to be aware of the location of the laboratory in relation to the sampling site, as well as the latest time of day that they are prepared to accept samples.

Other factors include deciding where to carry out analysis, i.e. in the laboratory or on site. Some analyses may be better performed on site, such as dissolved oxygen measurements, calibration of field measuring equipment, flow pumps and thermometers, etc. and sample treatments such as filtration. Some samples need to be split or subsampled. Where this is done, great care needs to be taken because samples are frequently very variable. Contingency plans need to be prepared for situations such as bad weather and vehicle breakdown. Field sampling sheets also need to be prepared. These can be filled in manually on paper forms or on a portable computer providing that the software has been properly validated. When designing field report forms it is important that the place, time and date of sampling, sampling conditions, any field measured variables, equipment used (with an inventory number), any necessary sample preparation and the name of the operator are included in the form. Practical difficulties, such as how many samples the field worker will need to carry, parking and access to the site also need to be considered.

Field safety issues

Field safety can have a bearing on the quality of data generated where field operators may be inclined to use a less than optimum procedure in order to protect themselves. This must be taken into account when writing the sampling procedure. For example, insisting on sampling water at chest height may deter some operators if the conditions in the water to be sampled are rough. Sampling from boats can be especially hazardous in rough weather. Even the 30 cm depth stipulation of the European Union's Bathing Water Directive can be difficult to comply with. When devising a plan, areas of risk may have to be borne in mind, including water depth and sampling conditions, currents, wildlife, traffic

and weather. Staff must always be provided with the appropriate protective equipment and SOPs should be developed with the safety of operators of paramount concern.

4.3.2 Field quality assurance

In spite of the difficulties involved in site work, QA is critical at this point. If a good, practical, field QA programme is put into operation, confidence in the data collected should be ensured (WHO/UNEP/VKI, 1997). All equipment must be kept clean and in good order, and records should be kept of all maintenance and of any irregularities that may affect the results. Conditions in the working area should not expose the operator to undue risk of any type.

Standardised and approved methodologies must be used at all times. If a method proves unworkable on site, then an alternative must be found quickly and agreed by all those involved. Operators must not change procedures without referral to the management procedure. Where unavoidable changes are made, for example, in bad weather, they must be fully documented. Nevertheless, a good sampling plan should make provisions for bad weather.

Prevention of sample contamination and losses

It is important that samples are protected from contamination and deterioration before their arrival in the laboratory. This can be ensured by using only recommended sample containers. Where reusable containers are used, it is essential that they have been cleaned properly and, if necessary, sterilised before use. Containers that have been sterilised must remain sterile until the sample is collected. The inner portion of the sample container should not be touched by the operator. If the seal on the bottle is broken (in the case of a commercially purchased microbiological sample bottle), or if the protective paper or foil has been lost from the top (home-made sampling containers), the bottle should be discarded.

Recommended preservation methods must be used. Where this involves chemical preservatives, the chemicals must be of analytical grade, and provided and tested for efficacy by the analytical laboratory.

Field measurements, such as pH and temperature, must be made on a separate subsample which is then discarded in order not to contaminate samples for interlaboratory analysis. Conductivity measurements should not be made with a sample that has been used previously for measuring pH, because potassium chloride from the pH probe may affect the conductivity reading.

All sample containers should be kept in a clean environment, away from dust, dirt and fumes. Petroleum products and fumes may contaminate samples with heavy metals and hydrocarbons. This can be a major problem on boats, where leaks and seepage of petroleum products are common. Samples must be stored in a cool box or portable refrigerator and transported to the laboratory as soon as possible. Cool boxes are more efficient if they contain some water.

Field Quality Control

Quality Control (QC) is an essential part of the field QA programme. It requires the collection of replicate samples to check the repeatability of sampling (see section 4.5.1), and the submission of field blanks and duplicates to check for contamination, handling and storage problems and other errors that may affect the results from the time of sampling to the time of analysis. The timing and frequency of these samples should be documented in the sampling plan.

4.4 Laboratory facilities

Except for any on-site analysis, analysis is usually performed in a laboratory. It is essential that any facilities are adequately equipped to deal with the analyses required and are convenient for the delivery of samples. This should have been ascertained before the start of the monitoring programme (see Chapter 2).

Small-scale organisations responsible for monitoring may find it more convenient to use outside facilities for analysis and sometimes for sampling. In these cases, the use of a laboratory belonging to an accreditation scheme is advisable and, moreover the laboratory should be inspected for compliance by an experienced member of the monitoring programme. An inspection should take into account the following features (ISO, 1984):

- Lines of communication between staff and management.
- Staff training and qualifications.
- Resources.
- Equipment maintenance and calibration.
- Standard Operating Procedures.
- Traceability of results.
- Sample handling and storage.

Where in-house facilities are used, it is essential that the monitoring work does not overload the laboratory. Resources (staff, space, equipment and supplies) must be sufficient for the planned workload. The laboratory must be well managed and must conform to all relevant health and safety guidelines. All analyses performed must be within the remit and expertise of the facility and SOPs must be in operation for all analyses (see Chapter 2).

4.4.1 Sample receipt and storage

Procedures for sample handling, transport and storage prior to analysis should ensure that the quality of the sample is not compromised. The condition of each sample and its storage location should be recorded along with its proposed analyses. If the sample is split, this must also be recorded. All samples must be identified uniquely with a number or code. It is important to ensure that the passage of a sample, and any associated paperwork, through the laboratory is fully documented and, therefore, traceable.

4.4.2 Reporting

The efforts of QA are directed ultimately towards ensuring that any data produced are suitable for their intended use; this applies to the results and any interpretations. The first stage in the reporting process is the examination of the results to see if they are fit to report (although raw data should have been checked prior to this stage). Results must be reported accurately and in a way that aids interpretation. To facilitate this, information may need to be included that has a bearing on interpretation, such as sampling conditions or the method of analysis. All data included must be checked by an experienced analyst with reference to site reports, calibration and QC data. Many laboratories have a system which requires the checking and countersigning of analytical reports (usually by the laboratory manager) to act as a safeguard against erroneous or misleading data leaving the laboratory. This type of system is only effective when conscientiously applied.

4.5 Analytical Quality Control

Analytical Quality Control consists of two elements: internal quality control (IQC) and external quality control (EQC). External quality control or inter-laboratory control is carried out periodically and checked by the laboratory responsible for the monitoring system. Internal quality control consists of the operational techniques used by the laboratory staff for continuous assessment of the quality of the results of individual analytical procedures. The focus is principally on monitoring precision, although accuracy is not ignored. It is necessarily part of the wider QA programme, but differs from it by the emphasis placed on quantifying precision and accuracy. Whereas QA strives to achieve quality by regulating procedures using management techniques, IQC focuses on the individual method and tests its performance against mathematically-derived quality criteria.

4.5.1 Internal quality control in the chemical laboratory

Internal quality control within the chemical laboratory comprises a variety of activities, some of which are described below (Briggs, 1996).

Choice of analytical method

A variety of different analytical methods are usually available for determining the concentration of any variable in a water sample. The choice of method is critical for ensuring that the results of the analysis meet the laboratory's requirements, because different methods have different precisions and sensitivities and are subject to different potential interferences. Consideration must be given to these parameters before a method is chosen. A number of standard methods are available for most of the analytical determinations involved in water quality monitoring, and in some cases the method is named in the regulations. These standard methods frequently include extensive validation data that allow the method to be evaluated easily. In addition, many methods are sanctioned by appropriate international or national organisations. It is important that any method selected meets the individual programme requirements. The performance of a method can be affected unpredictably by many factors.

Before any analytical method is put into routine use it is essential that it is properly validated. A minimum programme of validation includes a number of elements. One of these elements is the determination of linearity - the calibration point should be determined and if possible a linear response curve should be demonstrated. In addition, the limit of detection (the lowest concentration of the variable that can be distinguished from zero with 95 per cent confidence) should be determined. Within-and between-day coefficients of variation should be performed at three concentration levels to determine precision. Analysis of reference materials with known concentrations of the variable, or comparison analysis with existing methods in other laboratories, should be performed where possible.

Validity checking

After a method has been validated, found to be suitable and introduced into routine use in the laboratory, it is necessary to ensure that it continues to produce satisfactory results. Validity checks should be made on every batch of samples, or at frequent, regular intervals if batches are large or if testing is continuous. Validity checking is an extension of the checks carried out before the method was selected and is intended to confirm regularly the conclusions reached at that time.

Calibration check

If a calibration curve is being used, standard solutions should be analysed from time to time within the required range of concentration. The ideal calibration curve is linear within its most useful range, with a regression coefficient of 0.99 or greater. The response of the measuring equipment to the concentration of the variable in a standard solution (in terms of absorbance or some other parameter) should be recorded when it is expected that this parameter will be comparable from assay to assay. In addition, the deviation of individual calibration points from the line of best fit can be used to assess the precision of the calibration, which should be within the mean precision limits for the method.

Use of blanks

Method blanks and, where possible, field blanks should be analysed with each batch of samples. A method blank consists of reagent water, usually double-distilled water. A field blank is reagent water that has been bottled in the laboratory, shipped with sample bottles to the sampling site, processed and preserved as a routine sample and returned with the routine samples to the laboratory for analysis. The analysis of a blank should not yield a value higher than that allowed by the acceptance criteria. This procedure checks interference and the limit of detection of the assay.

Recovery checking

A specimen spiked with a known amount of the variable should be tested in each batch and the closeness of fit to the expected value calculated. In most cases this procedure provides a check on accuracy but, in assays where a variable is extracted from the original matrix (such as in many sample cleanup procedures used prior to chromatographic analysis), it can be used to monitor the extraction step. It is important that the matrix of the spiked specimen matches the real sample matrix as closely as

possible. Many laboratories use real samples with low natural values of the variable for this purpose, spiking them with known amounts of the variable and including both the spiked and natural samples in the same assay batch.

Precision and accuracy checks

Precision and accuracy checks are an extension of the validity checking described above. These checks allow the quality of the assay to be monitored over time using techniques such as control charting. The validity checks described above only allow acceptance or rejection of the assay data. Precision and accuracy checking should allow slow deterioration of data quality to be identified and corrected before data have to be rejected. This results in increased efficiency and reduced costs for the laboratory.

Control by duplicate analysis

Use of duplicate analysis as a method of precision checking has two distinct advantages: quality control materials are matrix-matched and the materials are readily available at no extra cost. Because the samples are analysed using the same method, equipment and reagents, the same bias will affect all results. Consequently, duplicate analyses are only useful for checking precision; they provide no indication of the accuracy of the analyses. Results from duplicate analyses can be used to calculate a relative range value, R, by using the equation:

$$R = \frac{(X1 - X2)}{(X1 + X2)/2}$$

where *X1* and *X2* are the duplicate results from an individual sample and *X1-X2* is the absolute difference between *X1* and *X2.* These values are then compared with the mean relative range values previously calculated for the assay during validation. The simplest method of assessment is to use the Upper Concentration Limit (UCL), where UCL = 3.27 × mean R value. When any value is greater than the UCL, the analytical procedure is out of control. This method, although statistically valid, provides no indication of deteriorating precision.

Precision control using pooled reference material

A more sophisticated approach is to use acceptance criteria based on warning and action limits. This method has the advantage of providing some monitoring of accuracy but is a viable control only if the material to be used will be stable in storage for sufficient time. The reference material is normally prepared by taking previously analysed samples with known concentrations of the variable under investigation, mixing them and aliquoting the resultant pool. The aliquots are then stored in readiness for analysis. A small sample of the aliquots is analysed to determine the mean concentration of the variable, and the standard deviation and the coefficient of variance at that concentration level. Data may be used only if they come from analysis that are in control. This approach requires that the new pool materials must be prepared before the old ones are finished.

A typical precision control exercise would involve the analysis of four aliquots from each pool in each of five assays, thus obtaining 20 results. The material from the pool should be analysed at several different times with different batches, because between batch variance is always slightly greater than within batch variance. Once 20 or more analyses have been made on this pool of material, the mean and standard deviations of the results are calculated. Any result that is more than three standard deviations from the mean is discarded and both of the statistics are recalculated. The mean is the “target” value and ideally, will be a close approximation of the true concentration of the variable in the reference material. The mean and standard deviation become the basis of the acceptance criteria for the assay method and may be used to draw up control charts.

At least three separate reference materials with different mean values of variable concentration should be in use at any one time in order to provide control of the analytical method across a range of concentrations. If precision is checked at only one concentration of the variable, it is impossible to detect whether precision is deteriorating at other concentrations. Use of several reference materials also allows their preparation to be staggered so that they become exhausted at different times. This assures greater continuity of control, because two or more old pools will still be in use during the first few assays of a new reference material.

Although the monitoring of accuracy by assessing deviation from the reference material mean (target value) is possible, care must be taken because the target value is only an approximation of the true value. As reference materials become exhausted and new ones are made, there will be a slow deterioration in accuracy. Accuracy can be safeguarded by regular participation in EQC exercises (see section 4.5.5) and by the use of certified reference materials.

Certified reference materials

Certified reference materials (CRMs) are matrix-matched materials with assigned target values and ranges for each variable, reliably determined from data produced by repeated analysis. Target and range values may be generated from data produced by several laboratories using different analytical methods or calculated from data obtained by the use of one analytical method (usually a reference method). Consequently, there may be bias in the target value. The target values assigned to each variable in the matrix in certified reference materials are generally very close to the true value. For some variables, however, there is an appreciable difference in bias between different analytical methods and this may lead to wide assigned ranges. When a laboratory is not using one of the reference methods the “all method” range may be so wide that it is practically meaningless. Certified reference materials are also only practical for variables that are stable in long-term storage.

Certified reference materials are prepared and checked under carefully controlled conditions and, as a result, they are costly to produce, correspondingly expensive to purchase and they may be difficult to obtain in some countries. Some authorities advocate the routine use of CRMs as precision control materials, but it is more cost effective to use them for the periodic checking of accuracy, in combination with a rigorous IQC programme.

Use of control charts

The principle of control charts is that IQC data can be graphically plotted so that they can be readily used and interpreted. Consequently, a control chart must be easy to use, easy to understand and easy to act upon. The Shewhart chart is the most widely used control chart (Shewhart, 1986). It is a graph with time (or assay batch) on the x-axis and the concentration of the variable in the reference material on the y-axis. Target, warning and action lines are marked parallel to the x-axis. Data obtained from precision control using reference materials (as described above) are usually plotted on a Shewhart chart. In this application, the target line is at the mean concentration of the variable for that specific pool of material and warning lines are placed at two standard deviations to either side of the target line. Provided the distribution is normal, 95 per cent of results from assays in control will fall between the two warning lines. Action lines are normally placed at three standard deviations to either side of the target line and 99 per cent of normally distributed results should be between the action lines.

In the regular use of a Shewhart chart, an aliquot from an appropriate reference material is analysed with every batch of samples and the measured concentration of the variable in the aliquot is plotted on the chart. Normally, no more than 1 in 20 consecutive results should fall outside the warning lines. If this frequency is exceeded, or if a result falls outside the action lines, the method is out of control.

The scatter of the assay results for the reference material around the target line provides an indication of the precision of the method, while the mean of the assay results relative to the target value indicates whether there is any bias (consistent deviation) in the results. If the analysis on one or more of the control specimens yields a result that it is outside the warning or action lines on the chart, the following action should be taken:

- A single result outside the warning lines should lead to careful review of data from that analytical batch and two or three subsequent batches.

- Results outside the warning lines more frequently than once every 20 consecutive analyses of control specimens should prompt detailed checking of the analytical method and rejection of the assay data.

- A result outside the action limits should prompt detailed checking of the analytical method and rejection of the assay data.

4.5.2 Internal quality control in the microbiology laboratory

Internal quality control in microbiology laboratories poses special problems of reproducibility due to the naturally wide variation in the number of organisms found between subsamples (see Chapter 8). Apart from method and field blanks (where the method blank should be sterile distilled water and the field blank should be a natural sample either guaranteed free of the test organisms or sterilised natural water), control samples should be analysed which are known to contain appropriate numbers of the micro-organisms that are normally sought. It is possible to purchase sets of freeze dried wild-type bacterial reference cultures for quality control and accreditation requirements. These cultures should be reconstituted and diluted with quarter strength Ringer's solution to give a suitable number of organisms similar to that which would normally be

seen in the natural samples. These cultures are expensive and therefore it is not feasible to use a new culture for every batch of samples or media. However, frequent subculture of reference strains is to be discouraged due to problems with contamination and mutation. This is a special problem with coliphage analysis, where mutation of the host species can prevent the detection of viral plaques. This problem can be solved by freezing down the cultures in glycerol broth and either storing in liquid nitrogen or a -70 °C freezer or, more conveniently, on commercially available plastic storage beads and freezing at -20 °C. Alternatively, some media companies supply standardised cultures in an easy to use form. These cultures may be qualitative or quantitative and have the advantage of eliminating the trial and error diluting of suspensions to achieve the desired count.

Shewhart charts can be used in water microbiology despite the problems of natural random variation. However, this means that wide control limits are necessary. For example, if the count reported for the first half of a duplicate sample is 11, then the 95 per cent confidence interval (CI) for the count of the second sample will be 3-23. Tables giving the CIs of counts are available in reference works on water analyses (HMSO, 1994). The microbiology laboratory should carry out several duplicate analyses regularly and plot the results on a control chart. Each half should be treated as a separate sample and analysed routinely. They should be inserted in the sample run in a random fashion without the knowledge of the analyst (if at all possible) and all results should be read by the same person. The first count should be recorded on the control sheet, and the corresponding CI for the second count entered. The second count is then recorded along these figures. If this count falls outside the CI, this fact should be highlighted. If a Shewhart chart is made up of these results, then any trend can be identified. If, over a period of time, the second count falls outside the CI for more than 95 per cent of the time, the reason should be investigated. As with the use of duplicate samples in chemistry, this approach keeps a check on precision and not on accuracy.

In addition to blanks and a manufactured control, the use of a known wild positive can be included. This can be chosen from the last batch of samples run. However, there can be problems with this, due to alteration in bacterial numbers over time and storage.

All prepared media should be checked for performance and sterility and identified by batch reference number. Both negative and positive control strains of bacteria should be included. Manufacturers of dried media will usually recommend control strains if requested. Where a medium is meant to inhibit the growth of a particular organism, this should also be tested.

4.5.3 Summary of an internal quality control programme

A summary of the IQC programme recommended by the GEMS/Water programme is given below. This programme offers a simple but effective introduction to IQC and is described in more detail in the *GEMS/Water Operational Guide* (WHO, 1992). For each variable the following should be applied:

- For chemical variables, analyse five standard solutions at six different known concentrations covering the working range to develop a calibration curve or, when a calibration curve already exists, analyse two standard solutions at different known concentrations covering the working range to validate the existing calibration curve.

• Analyse one method blank per set of 20 samples.

• Analyse one field blank per set of samples.

• Analyse one duplicate of a sample chosen at random from each set of up to 20 samples.

• Analyse one specimen that has been spiked with a known amount of the variable as a recovery check. This specimen should have a matrix similar to those of the samples being processed.

4.5.4 Remedial action

If any of the QC procedures indicate that a method is out of control or that a problem exists, corrective action must be taken. The main checks to make are calculations and records, standard solutions, reagents, equipment and QC materials (Table 4.1).

Table 4.1 Checks to be carried out when a problem is detected with an analytical method

Problem area	Checks
Calculations and records	Check calculations for transposition of digits or arithmetic errors; confirm that results have been recorded in the proper units and that any transfer of data has been made correctly
Standard solutions	Check the standard solutions that are used for calibrating equipment; check their storage conditions and shelf-life (an old solution may have deteriorated or a new one made up incorrectly)
Reagents and media	Check for deterioration of old products; check QC records to see if new reagents performed correctly and if they were properly prepared; check their storage conditions and shelf-life
Equipment	Check calibration and maintenance records for all relevant dispensers and measuring equipment where a method is out of control; items such as automatic pipettes, balances and spectrophotometers should be checked regularly and recalibrated as necessary Ascertain that equipment is being properly used; check that any QC material has not deteriorated and is properly stored; run analyses on several aliquots to determine whether the concentration of the variable remains within the allowed deviation from the target value and close to the mean of the last 20 determinations

Source: Briggs, 1996

4.5.5 External quality control

External quality control is a way of establishing the accuracy of analytical methods and procedures by comparing the results of analyses made in one laboratory with the results obtained by others conducting the same analysis on the same material. This is usually accomplished by one laboratory sending out sets of samples, with known and unknown concentrations of variables, to all of the specified laboratories. Each participant analyses the samples for the specified variables and reports the results to the reference laboratory

(Box 4.1). The results from all participating laboratories are collated by the organisers of the EQC programme and then subjected to detailed statistical analysis. A report to each laboratory is generated, giving a target value for the reference sample or samples (usually consensus mean or median), a histogram illustrating distribution of results for each material, and an individual performance score relating the individual laboratory results to the target value. The calculations for performance indicators are often quite complex because multiple specimens have to be considered and the method variance varies with the concentration of the variable. However, the general principle of providing a method of performance comparison remains the same in all EQC exercises.

Box 4.1 External Quality Assurance: the experience of a microbiology laboratory

The Unit of Microbiology, Faculty of Medicine, University Rovira i Virgili (Reus, Spain) participates in an EQA on microbial recovery where each participating laboratory analyses an external sample and the results from all participating laboratories are analysed statistically.

A critical element of the EQA is that the test sample should be processed in an identical manner to routine samples. If an analyst is aware that an external sample is to be processed the exercise is viewed as a test of their competence and modifications may be made to routine procedures in order to enhance recovery. Analysts may also use the exercise to test potential new methodologies, but this is not the purpose of EQA and statistically unreliable results may be obtained if new methods are deliberately applied.

Although relatively expensive, properly operated EQA exercises can be of great benefit to participating laboratories because they can identify failures in internal quality control and, if undertaken over a period of time, laboratories can use them to evaluate regularly the performance of their methods. Corrective measures can then be applied whenever the methods are found to be producing poor results.

The key issue identified in participating in such EQA exercises was that they must be carried out anonymously so that the samples are dealt with in exactly the same manner as routine samples.

External quality control reports should indicate clearly whether performance is satisfactory or not. If it is not satisfactory, two general actions must be taken. First, the analysis at fault must be examined to determine the cause of poor performance. Secondly, the IQC programme that allowed the deterioration to progress unchecked must be closely examined to establish where inadequacies exist. Both must be corrected.

The general objective of EQC is to assess the accuracy of analytical results measured in participating laboratories and to improve interlaboratory comparability. Wherever possible, laboratories should participate in EQC programmes for each variable that is analysed routinely. This is only worthwhile where IQC is also part of a laboratory's normal procedures. Participation in relevant EQC programmes, and maintenance of adequate performance in those programmes, is often a requirement for laboratory accreditation.

The organisation of an EQC exercise is expensive. Large quantities of stable reference materials must be prepared, these materials must be transported to the participating

laboratories, data must be analysed and detailed reports on performance must be prepared. Participating laboratories are usually charged for the service provided.

4.6 Elements of good practice

• Monitoring programmes should include appropriate QA which does not infringe on health and safety and which covers the integrity of all observation, interviews, field sampling and water quality analyses as well as data input, analysis and reporting.

• A QA manager should be appointed who audits all aspects of the operation regularly with special regard to procedures, traceability of the data and reporting.

• Essential elements of QA programmes include:

• The writing and implementation of a Quality Manual and SOPs. All SOPs should be overhauled regularly and updated as necessary, and any deficiencies should be reported and appropriate remedial action taken.

• SOPs should include maintenance and updating of inventories and catalogues; methodologies for all major equipment, all sampling and analytical procedures; sample receipt, screening and storage; and reporting.

• Σαμπλεσ σηουλδ βε ρεγιστερεδ ον αρριϖαλ ατ τηε λαβορατορψ. Τηε αππλιεδ λαβορατ ορψ προχεδυρεσ σηουλδ χονφορμ το τηε ΣΟΠσ δεφινεδ ατ τηε λαβορατορψ. Ωηερε ποσσ ιβλε, αλλ αναλψτιχαλ προχεδυρεσ σηουλδ φολλοω δεφινεδ ΙΣΟ ορ Αμεριχαν Πυβλιχ Ηε αλτη Ασσοχιατιον (ΑΠΗΑ) προτοχολσ. Αλλ εθυιπμεντ σηουλδ βε χαλιβρατεδ ρεγυλαρλ ψ ανδ τηε οπερατιοναλ προχεδυρεσ συβμιττεδ το θυαλιτψ χοντρολ σταφφ ιν ορδερ το γυ αραντεε τραχεαβιλιτψ οφ τηε δατα.

• The programme should be evaluated periodically, as well as whenever the general situation or any particular influence on the environment is changed.

4.7 References

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