Chapter Five
Laboratory Analysis

5.1. Introduction

The aim of laboratory analyses is to obtain accurate and precise data in a safe environment. A framework for designing an analysis program is given in Figure 5.1. A checklist of important considerations is given in Table 5.1. For a typical laboratory request form see Appendix A6.2.

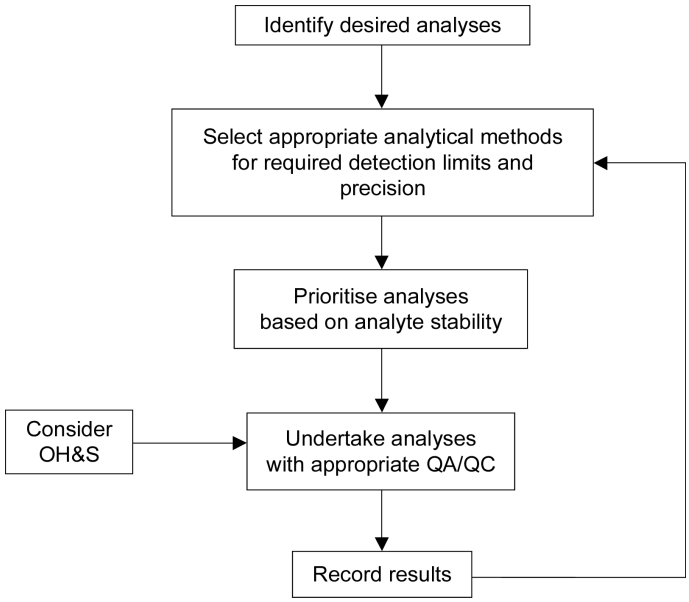

Figure 5.1. A framework for designing an analysis program

5.2. Analytes

The particular substances to be analysed (analytes) are the focus of the monitoring program. They may have been identified in generic terms during the study design, but now the individual compounds need to be decided on, and possible methods of determination need to be considered before planning the laboratory program. The analytes will also determine many of the decisions involved in laboratory analyses: for example, how to obtain good quality data (method and equipment), how to protect the health and safety of workers, and how much this stage of the monitoring program will cost.

Table 5.1. Checklist for undertaking laboratory analyses

1. Have the analytes been clearly stated?
2. Have appropriate analytical methods been identified?
   (a) Will analytical methods cover the range of concentrations expected?
   (b) Will analytical methods detect the minimum concentration of interest?
   (c) Will the analytical methods have sufficient accuracy and precision?
   (d) Will the substances be processed within the samples' storage life?
3. Does the laboratory have the appropriate equipment to undertake the analytical method chosen?
4. Are laboratory facilities (water supply, air supply, environment) suitable for the planned analyses?
5. Do the laboratory staff have the expertise, training and competence to undertake the planned analyses?
6. Has a laboratory data management system been established? Does the system
   (a) track samples and data (chain of custody)?
   (b) have written data entry protocols to ensure correct entry of data?
   (c) enable associated data to be retrieved, e.g. nutrient concentrations and flows to calculate nutrient loads?
   (d) have validation procedures to check accuracy of data?
   (e) have appropriate storage and retrieval facilities to prevent loss of data and enable retrieval (for at least three years) based on current and unexpected information needs?
   (f) Are procedures in place to ensure information has reached the user?
7. From the documentation, can this information be seen:
   (a) how results were obtained?
   (b) that samples had unique identification?
   (c) who the analyst was?
   (d) what test equipment was used?
   (e) the original observations and calculations?
   (f) how data transfers occurred?
   (g) how standards were prepared?
   (h) the certified calibration solutions used, their stability and storage?
8. Has a laboratory quality assurance plan been developed? Does the plan specifically cover:
   (a) operating principles?
   (b) training requirements for staff?
   (c) preventative maintenance of laboratory infrastructure and equipment?
   (d) the requirements of the laboratory data management system?
   (e) procedures for when and how corrective actions are to be taken?
   (f) allocation of responsibility to laboratory staff?
   (g) all documentation to maintain quality at specified levels?
   (h) quality control procedures to minimise analysis errors?
   (i) quality assessment procedures to determine quality of data?
9. Are all protocols for preparing and analysing samples written and validated?
10. Are standard methods being used? If variations of standard methods or non-standard procedures are used, procedures must be technically justified, with documentation of the effects of changes.
11. Have analytical methods' accuracy, bias and precision been established by:
    (a) analysis of standards?
    (b) independent methods?
    (c) recovery of known additions?
    (d) analysis of calibration check standards?
    (e) analysis of reagent blanks?
    (f) analysis of replicate samples?

Table 5.1. continued

12. Do operation procedures specify instrument optimisation and calibration methods, and do these cover:
    (a) preventative maintenance?
    (b) specific optimisation procedures?
    (c) resolution checks?
    (d) daily calibration procedures?
    (e) daily performance checks?
13. With respect to laboratory QA/QC:
    (a) have control charts for laboratory control standards, calibration check standards, reagent blanks and replicate analyses been established?
    (b) does the laboratory participate in proficiency testing programs?
    (c) does the laboratory conduct unscheduled performance audits in which deviations from standard operating procedures (protocols) are identified and corrective action taken?
14. Have all reasonable practical steps been taken to protect the health and safety of laboratory staff?
    (a) have hazards been identified?
    (b) have laboratory staff been educated about hazards?
    (c) have risk minimisation plans been prepared?
    (d) have staff been trained to ensure safe work practices?
    (e) are staff appropriately supervised?
    (f) are staff insured?

5.3. Choice of Analytical Methods

The selection of an analytical method for waters, sediments or biota will largely depend on the information and management needs of those undertaking the investigation, and on the analytes themselves. However, limitations such as the financial resources available, laboratory resources, speed of analyses required, matrix type and contamination potential, are also important factors.

The choice of an appropriate analytical method is based on four considerations:

- the range of concentrations of the analyte that need to be determined. Detection limits are method specific and the lowest concentration of interest will need to be specified.
- the accuracy and precision required. All results are only estimates of the true value and the greater the accuracy and precision required the greater the analytical complexity and cost.
- the maximum period between sampling and analysis. On-the-spot field analysis may be required, depending on the use to be made of the data.
- Where several methods can achieve the above requirements, the ultimate choice may be dictated by familiarity with the method and/or the availability of necessary analytical instrumentation.

Appropriate procedures for both chemical and biological analyses can be found by reference to accepted published procedures such as *Standard Methods for the Examination of Water and Wastewater* (APHA 1998) or USEPA sampling and analysis methods (Keith 1991), or the most recent editions of these. Methods that cover water, sediment and biological analyses are available on the USEPA web site, http://www.epa.gov. Detailed reviews or descriptions of methods are available in standard texts; however, a good general text with methods encompassing all the parameters covered in this section is Rayment and Higginson (1992).

Methods for marine water analysis are found in Grasshoff et al. (1999) and Parsons et al. (1985). A summary of analytical methods and associated references is provided in Table 5.2. Methods for toxicity testing are not included, but have been summarised in section 3.5 in Chapter 3. Standard methods are updated regularly; however with the pace of research, many good non-standard methods are available that have not yet been included. Their use is acceptable, provided that justification for

their choice is given and that their performance can be demonstrated through the analysis of standard reference materials or other quality control procedures.

Aspects or details of both standard and non-standard procedures may require evaluation or modification for use in Australian or New Zealand conditions. Some of these modifications are well documented, but others either are not recorded or are recorded in commercial methods that are not available to the authors of the Monitoring Guidelines.

Table 5.2. Summary of analytical methods for physical and chemical parameters

Analyte	Methods	References*
Physical		
Clarity	Secchi Disk	APHA (1998)
Temperature	Thermometer, electronic data logger, thermistor	
Flow rates	Acoustic Doppler current profiler, assorted methods	USEPA (1982), RD Instruments (1989)
Depth	Acoustic Doppler current profiler, depth sounder	RD Instruments (1989), EPA (1992)
Colour	Colorimetry	APHA (1998), USEPA (1983)
Suspended solids	Gravimetry	APHA (1998), USEPA (1983), AS (1990)
Turbidity	Nephelometry, light scattering	APHA (1998), USEPA (1983)
Conductivity	Instrumental	APHA (1998)
Gross contamination	Floatables, solvent-soluble floatable oil and grease	APHA (1998)
Chemical		
pH, alkalinity, acidity	Electrometry and titration	APHA (1998), USEPA (1983)
Salinity	Electrical conductivity, density, sensors, titration	APHA (1998), Grasshoff et al. (1999), Parsons et al. (1985)
Dissolved oxygen	Iodometry, oxygen electrode, Winkler method	APHA (1998), USEPA (1983)
Biological oxygen demand	Incubation	APHA (1998), USEPA (1983)
Chemical oxygen demand	Reflux, titrimetry, colorimetry	APHA (1998), USEPA (1983)
Organic carbon	Combustion infrared, persulfate UV oxidation	APHA (1998), USEPA (1983)
Metals	ICPAES, ICPMS, AAS, etc., plus specialist methods for Al, Hg, As, Se, Cr(VI), speciation	APHA (1998), USEPA (1983, 1994c), USEPA (1996c) (Hg); USEPA (1996d) (As)
Ammonia	Ammonia electrode, titrimetry, colorimetry	APHA (1998), USEPA (1983)
Nitrate, nitrite	Colorimetry, ion chromatography, etc.	APHA (1998), USEPA (1983), Grasshoff et al. (1999), Parsons et al. (1985)
Total Kjeldahl nitrogen	Colorimetry, potentiometry	APHA (1998), USEPA (1983)
Carbonate, bicarbonate, CO_2	Titrimetry	APHA (1998), Parsons et al. (1985)
Hardness	Titrimetry	APHA (1998)
Phosphorus	Colorimetry	APHA (1998), USEPA (1983)

Table 5.2. continued

Silica	AAS, Colorimetry, ICPAES	APHA (1998), USEPA (1983), Grasshoff et al. (1999), Parsons et al. (1985)
Cyanide	Titrimetry, colorimetry, cyanide-selective electrode	APHA (1998), USEPA (1993)
Sulfur compounds	Assorted	APHA (1998), USEPA (1983)
Chlorine	Iodometry, amperometry	APHA (1998), USEPA (1983), Grasshoff et al. (1999), Parsons et al. (1985)
Chloride	Colorimetry, titrimetry, IC, potentiometry	APHA (1998), USEPA (1983)
Chlorophyll	Fluorimetry, spectrophotometry	APHA (1998), Parsons et al. (1985)
Oil and grease	Assorted	APHA (1998), USEPA (1983)
Surfactants	Spectrophotometry, etc.	APHA (1998), USEPA (1983)
Phenols	Assorted	APHA (1998), USEPA (1983)
Organochlorine compounds	GC ECD, GC-MS	APHA (1998), USEPA (1983, 1995, 1996e)
Organophosphate pesticides	GC	APHA (1998), USEPA (1983, 1996e)
Carbamate pesticides	HPLC	USEPA (1996e)
Chlorinated phenoxyacid herbicides	GC	APHA (1998), USEPA (1996e)
Dioxins	GC-MS	USEPA (1983, 1996e)
PAHs	HPLC, GC, GC-MS	APHA (1998), USEPA (1996e)
Radioactivity	Counting	APHA (1998)
Biological		
Coliform bacteria	Enumeration, enzyme assays	APHA (1998)
Bioaccumulation	Standard analytical procedures	
Toxicity testing	Bioassays using fish, invertebrates, algae and bacteria	See section 3.5.2

*for APHA (1998), USEPA volumes and AS (1990), use these or the most current editions

Considerable information on the modes of action of the various parameters, their effects on human health, and guidelines on tolerable concentration limits is given in the Water Quality Guidelines (ANZECC & ARMCANZ 2000). The onus is on the practitioner to ensure that environmentally responsible methods are chosen whenever possible. Waste disposal, in particular, must be considered when deciding on a suitable method. It may not be necessary to use an elaborate and expensive method if a more up-to-date, cost-effective and less environmentally damaging technique (such as a field testing procedure) may provide the information at the required level of accuracy.

Before analyses are undertaken, the monitoring team and the end-users of the data should confirm that the chosen laboratory has the appropriate equipment, expertise and experience to undertake the analytical method chosen, as well as an adequate quality assurance program. If the monitoring team plans to send samples to external laboratories, it is recommended that those accredited by the National Association of Testing Authorities (NATA) be used wherever possible. Accreditation guarantees appropriate standards of laboratory organisation and of QA/QC, but not necessarily accurate results.

Results can be reported in a variety of different units such as g/L, ppm, molarity, number of organisms, and so on, but this can cause a great deal of confusion and wasted effort when results from different sources are combined or compared. It is recommended that a system of consistent units is

adopted such as in the Water Quality Guidelines, namely those based on mass/L or mass/kg as appropriate.

5.4. Data Management

When samples are delivered to the laboratory for analysis, it is essential that the laboratory staff log the samples into the laboratory register or record system, and give each a unique identification code. This becomes part of the chain of custody of the sample.

The record system needs to:

- provide a traceable pathway covering all activities from receipt of samples to disposal;
- allow retrieval, for a period of at least three years, of all original test data within the terms of registration.

5.4.1. Data Storage

5.4.1.1. System Design Considerations

Water quality data are expensive to collect, requiring a substantial investment of time and money, so they must made as useable and useful and retrievable as possible by careful systematic storage. The sheer volume of data accumulated by any monitoring program, after just a few years of monitoring, dictates that computer-based data management systems must be the basis for data storage and management.

A data management system should have:

- reliable procedures for recording results of analysis or field observations;
- procedures for systematic screening and validation of data;
- secure storage of information;
- a simple retrieval system;
- simple means of analysing data;
- the flexibility to accommodate additional information, e.g. analytes, sites, etc.

The needs of the user are the most important consideration when a water quality database is being designed. Its designers must consider:

- the scope of the data to be stored — the sources, numbers of samples (and for each one its sample number, type, site, time or date of collection, etc.), number of database fields, descriptive notes, confidence categories, analytes, analysis types, number of records;
- issues caused by multiple sources of data — validation and standardisation procedures, transfer formats, confidence ratings;
- quality assurance and quality control (risk/confidence levels) — analytical precision, validation procedures;
- linkage to flow or tides;
- documentation — standard methods of analysis, validation procedures, codes;
- how the data will be accessed by users — on-line at the same time as it is generated (real-time), on-line retrieval, data retrieval request-based system, categories of data (macro design);
- the kinds of data analysis support required — statistical, graphical, trend analysis, regression analysis.

The Australian and New Zealand Land Information Council (ANZLIC) has a national standard for setting up meta-databases; see their web site, www.anzlic.org.au/asdi/metaelem.htm.

5.4.1.2. Data Tracking

If data are to be the basis for legal proceedings at some time in the future, a chain of custody is particularly important (see also section 4.6.1). In this context, the laboratory may be asked these questions:

- how was each sample labelled to ensure no possibility of mix up or substitution?
- how were the data identified to ensure no mix up or substitution?

The laboratory record system already mentioned will ensure the integrity of the sample from collection to final analysis with respect to the variables of interest. In it, all data need unique identification codes. Then, chain of custody documentation ensures that the questions above can be answered.

5.4.1.3. Screening and Verification

Data entry protocols must be developed to ensure that the entry of data is accurate. Data from instruments should be electronically transferred to the database where possible to prevent transcription errors.

5.4.1.4. Harmonisation of Data

Harmonised data are data that can be used or compared with data from other data sets in comparable units of measurement or time frames. For example, if nutrient loads are to be calculated, concentration data and flow data must be collected at the same location at the same time. To ensure that data are harmonised and can be usefully compared, the monitoring team must consider making additional measurements.

5.4.1.5. Retrieval and Sharing of Data in Databases

A wide range of individual databases has been developed, often associated with the operations systems of particular authorities (water supply, waste water management, storage management). Some of these systems have been costly to update for use with new computer technologies, and have been incompatible with other databases, resulting in difficulties in transferring data.

There has been substantial growth in electronic transfer and on-line access to data in recent years, requiring that databases be standardised. There are a number of commercially available databases, e.g. dB4®, dB5®, ACCESS® and FoxPro®. The adoption of these databases by industry, and the suppliers' commitment to their periodic upgrading to exploit new computer technologies and software developments, ensure that they will continue to have relevance and utility. The choice of a particular database depends on the types and intended uses of the data, and the type and compatibility of the computer hardware and software. The data should be available and able to be shared with other databases for years to come.

5.4.2. Laboratory Data Reporting

Where separate laboratory reports are provided as part of a monitoring program, these should include:

- the laboratory name and address;
- tabulation of samples and analysis data;
- identification of the analytical methods used;
- date of analysis and name of technician or chemist;
- a quality assurance statement.

These details are sometimes reproduced in full, for all relevant samples, in the appendixes of a primary report; otherwise the most appropriate data are abstracted and listed within the body of the report. Further details on reporting are provided in Chapter 7.

5.5. QA/QC in Laboratory Analyses

The objective of a quality assurance and quality control program in a laboratory is to minimise errors that can occur during sub-sampling and analytical measurement and to produce data that are accurate, reliable and acceptable to the data user. Therefore, the QA/QC procedures are designed to prevent, detect and correct problems in the measurement process and to characterise errors statistically, through quality control samples and various checking processes.

5.5.1. Traceability of Results

Traceability of analytical results from the laboratory report back to the original sample is an essential component of good laboratory practice, and is a prerequisite for accreditation of analytical laboratories.

Apart from its chain of custody details for each *sample*, the laboratory record system must include the following information for each *analysis*:

- identity of the sample analysed;
- identity of analyst;
- name of equipment used;
- original data and calculations;
- identification of manual data transfers;
- documentation of standards preparation;
- use of certified calibration solutions.

5.5.2. Laboratory Facilities

It goes without saying that the laboratory facilities and environment must be clean, with appropriate consideration of occupational health and safety issues. In addition, regular checking for airborne contamination is desirable; it can enter through air conditioning systems or be generated internally from users of the laboratory. Deionised water is the most extensively used reagent in the laboratory and it must be maintained at the appropriate standard required to conduct analyses. The electrical conductivity of the deionised water should be monitored continuously or on a daily basis, with the water being checked regularly for trace metals and organic compounds.

5.5.3. Analytical Equipment

All equipment and laboratory instruments should be kept clean and in good working order, with up-to-date records of calibrations and preventative maintenance. Repairs to equipment and instruments should be recorded, as should details of any incidents that may affect the reliability of the equipment.

5.5.4. Human Resources

All staff undertaking analyses must be technically competent, skilled in the particular techniques being used and have a professional attitude towards their work. Thus staff will need to be trained in all aspects of the analyses being undertaken.

Before analysts are permitted to do reportable work, they must demonstrate their competence to undertake laboratory measurements. As a minimum, they should be able to show they can adhere to a written protocol and that their laboratory practices do not contaminate samples. They should demonstrate their ability to work safely in the laboratory and to use the prescribed methods to obtain reproducible data that are of acceptable accuracy and precision.

5.5.5. QA/QC in Analytical Protocols

Laboratories undertaking analyses must fully document the methods used. Methods must be described in sufficient detail that an experienced analyst unfamiliar with a method can reproduce it and obtain acceptable results.

Laboratory staff should be aware that is important to adhere strictly to analytical protocols. They should appreciate the critical relevance of rigorous quality control and assurance in the laboratory. Proper laboratory practice is codified in the requirements of registration authorities such as the National Association of Testing Authorities (NATA). Laboratories holding registration from this and similar organisations will be familiar with the effort required to achieve and maintain a facility with creditable performance standards.

All laboratories must have a formal system of periodically reviewing the technical suitability of analytical methods. If standard methods are used, it is not enough to quote the standard method; any variation of the standard method must be technically justified and supported by a documented study on the effects of the changes.

Measurement errors can be divided into two types: random and systematic.

- *Random errors* affect the precision of the results; that is, the degree to which data generated from repetitive measurements of a sample or samples will differ from one another. Statistically this is expressed as the standard deviation for the replicate measurements of an individual sample and the standard error for replicate measurements of a number of samples. It may also be given as the coefficient of variation; that is, the standard deviation divided by the mean expressed as a percentage. This is often referred to as repeatability or reproducibility. Sources of random error are spurious contamination, electronic noise, uncertainties in pipetting and weighing, etc.
- *Systematic errors* or biases result in differences between the mean and the true value of the analyte of concern (accuracy). Systematic errors can only be established by comparing the results obtained against the known or consensus values. Sources of systematic error are reagent contamination, instrument calibration, method interferences, etc.

The principal indicators of data quality are its *bias* and *precision*. Bias is a measurement of systematic error, and can be attributed either to the method or to the laboratory's use of the method. Precision is the amount of agreement between multiple analyses of a given sample (APHA 1998). When combined, bias and precision are expressed as *accuracy*; that is, the nearness of the mean of a set of measurements to the 'true' value (APHA 1998). The data can be referred to as being accurate when the bias is low and the precision is high (Figure 5.2).

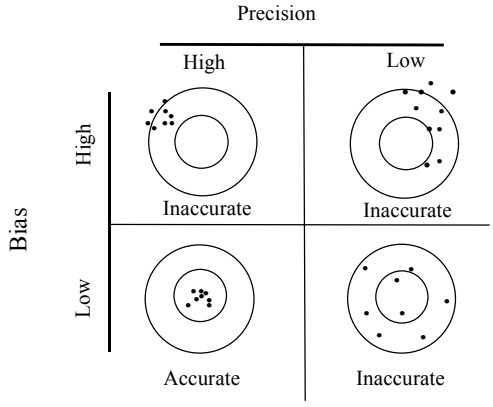

Figure 5.2. Illustration of accuracy in terms of bias and precision (modified from APHA 1998)

Quality assessment is the process of using standard techniques for assessing the accuracy and precision of measurement processes and for detecting contamination. The accuracy of analytical methods, for example, can be established by:

- analysis of reference materials,
- inter-laboratory collaborative testing programs in which it is assumed that the consensus values for analytes are true;
- performance audits;
- independent methods comparisons;
- recovery of known additions;
- calibration check standards;
- blanks;
- replicate analyses.

Similarly the appropriateness of sample storage and preservation procedures can be assessed by inter-laboratory collaborative testing programs. Statistically true values have confidence intervals associated with them, and sample measurements that lie within the confidence intervals will be considered to be accurate.

5.5.5.1. Analysis of Certified Reference Materials and Internal Evaluation Samples

Certified reference materials are materials of known concentrations which have a matrix similar to that of the sample being analysed. The accuracy of laboratory methods and procedures can be established by comparing the values for an analyte in the certified reference material against the results obtained by the laboratory for the same analyte. Results within the confidence limits specified for the certified reference material are deemed acceptable. The National Institute of Science and Technology (USA), Canadian Council for Reference Materials (Canada), International Atomic Energy Commission (Europe), Institute for Reference Materials and Measurements (Belgium) and the National Institute of Environmental Standards (Japan) provide a comprehensive range of certified reference materials.

'Internal evaluation samples' is a general term for samples (made up by an outside source or by the laboratory staff) that have a known analyte concentration, e.g. certified reference materials. The acceptable range of measurement (recovery and precision) is determined for these samples, and analysts are expected to be within this range in all their analyses of these evaluation samples.

5.5.5.2. Proficiency Testing Programs (Interlaboratory Comparisons)

Interlaboratory comparison of unknown samples is used for testing instrument calibration and performance and the skills of the operator. Testing authorities frequently sponsor these programs. Generally only a modest degree of sample preparation is required, probably to restrict the range of sources of variance between laboratories. An individual laboratory compares its results against the consensus values generated by all the laboratories participating in the program, to assess the accuracy of its results and, hence, the laboratory procedures. However, it should be noted that the consensus values can be wrong, and there should be a known value from the authority conducting the proficiency program. Results within the confidence limits specified for the unknown samples are deemed acceptable.

5.5.5.3. Performance Audits

During performance audits, unscheduled checks are made to detect deviations from standard operating procedures and protocols, and to initiate corrective action.

5.5.5.4. Independent Methods Comparison

The accuracy of analytical procedures can be checked by analysing duplicate samples by two or more independent methods. For the methods to be independent they must be based on different principles of analysis. For example, the determination of iron in water can be checked by physical or chemical

principles, e.g. atomic absorption spectrometry (light absorption by atoms) vs. anodic stripping voltametry (electrochemical reduction).

Bias in methods (i.e. interferences, insensitivity to chemical species, etc.) can cause two methods to give different results on duplicate samples. The average values obtained by the methods are compared using a Student's *t*-test (see Appendix section A5.1.6).

5.5.5.5. Recovery of Known Additions

By spiking a test sample with a known amount of analyte, it is possible to estimate the degree of recovery of the analyte and hence the accuracy of the method used. Spiking is one way of detecting loss of analyte. It is assumed that any interference or other effects that bias the method will affect the analyte spike and the analyte in the test sample in similar ways. Hence acceptable recoveries of the spike confirm the accuracy of the method.

This approach may be invalid if:

- the chemical species that are added are different to the native chemical species in the sample and therefore undergo different processes and interferences. An example is the spiking of marine biological samples with AsO_3^{2-}, when the native arsenic is present as arsenobetaine, $CH_3As^+CH_2COO^-$.
- the interference is dependent on the relative concentrations of the analyte and interferent. The addition of a spike will change this dependence and hence the magnitude of the interference.
- the interference is constant, regardless of the analyte concentration. Recoveries can be quantitative but analysis of the native analyte may have large errors.

5.5.5.6. Calibration Check Standards

Standard curves (i.e. calibration curves) must be verified daily by analysing at least one standard within the linear calibration range. This ensures that the instrument is giving the correct response and reduces the likelihood of concentrations in samples being under- or overestimated.

5.5.5.7. Blanks

Blanks should be incorporated at every step of sample processing and analysis. However, only those blanks that have been exposed to the complete sequence of steps within the laboratory will routinely be analysed, unless contamination is detected in them. Blanks incorporated at intermediate steps are retained for diagnostic purposes only, and should be analysed when problems occur, to identify the specific source of contamination.

In principle, only field blanks need to be analysed in the first instance, because they record the integrated effects of all steps. However, a laboratory will normally wish to test the quality of its internal procedures independently of those in the field, so laboratory procedural blanks will usually be included in a suite for analysis, in addition to field blanks.

Blanks cannot be used to detect loss of analyte; they are useful only to detect contamination. They are particularly useful in detecting minor contamination, where the superimposition of a small additional signal on a sample of known concentration may not be evident in the statistical evaluation of analytical data. In other words, blanks are more sensitive to contamination.

If any blank measurement is further than three standard deviations from the mean, or if two out of three successive blanks measurements are further than two standard deviations from the mean, the analyses should be discontinued and the problems identified and corrected.

5.5.5.8. Duplicate Analyses

Duplicate analyses of samples are used for assessing precision. At least 5% of samples should be analysed in duplicate.

5.5.6. QA/QC in Biological Analyses

For biological analyses, quality control procedures are designed to establish an acceptable standard of subsampling, sorting and identification.

5.5.6.1. Subsampling and Sorting

For quality control of subsampling and sorting, a sub-sample equivalent in size to the original subsample should be sent to an independent group, for checks. With macroinvertebrates, the data from the two sub-samples are analysed to compare community composition and structure. The analysis compares the ratios of numbers of taxa in each subsample, and the Bray–Curtis dissimilarities in each subsample. After a sample has been sorted, the remainder is checked for macroinvertebrates that have been missed. Checking continues until >98% of the total number of macroinvertebrates in the sub-sample have been consistently removed.

5.5.6.2. Identification

In general all organisms should be identified against taxonomic keys. If keys are not available, preserved samples should be sent to other laboratories that regularly identify similar samples.

Staff who must identify biological specimens should be trained in the use of keys, and their proficiency should be tested before they are given responsibility for the analysis of samples. For example, in the AUSRIVAS program, new staff identify organisms to family level in samples, and experienced staff check the same samples. The two lists of families are compared and discrepancies are discussed until the new staff understand their errors. The new staff continue to check samples, but fewer samples are cross-checked by the experienced staff as the new staff improve. As many as two samples in 10 may be cross-checked in the early stages of training, but later this drops to two samples in 50. New staff are considered proficient once their error rate at identification to family level is less than 10%.

5.5.7. QA/QC in Ecotoxicity Testing

In ecotoxicity testing, any variability in the test organisms or their health is critical to the quality of the ecotoxicity results. For this reason, standard protocols specifying the life stage and health of an organism are essential. Quality assurance procedures in ecotoxicity tests include criteria for test acceptability, appropriate positive and negative controls, use of reference toxicants, and water quality monitoring throughout the bioassays.

5.5.7.1 Test Acceptability Criteria

All ecotoxicity tests should have criteria for test acceptability. This is particularly important when the test is based on organisms collected in the field, which may vary in their response from season to season. For example, in growth inhibition tests with microalgae, the growth rates of the control group of organisms must exceed a pre-defined daily doubling rate with less than 20% variability. Similarly, in acute tests with invertebrates and fish, at least 90% of the untreated control organisms must be alive after 96 hours. Fertilisation or reproduction tests usually specify an acceptable fertilisation rate, abnormality rate or number of offspring produced. If these criteria are not met, the tests are invalid and must be repeated.

5.5.7.2. Negative Controls

All toxicity tests require the use of controls to compare the responses of the organisms in the presence or absence of toxicant. Negative controls can be uncontaminated seawater or freshwater used as diluent in the toxicity tests, with water quality characteristics similar to those of the test water. For sediment tests, negative controls include uncontaminated sediments that have particle sizes, organic carbon and sulfide contents similar to those of the test sediment.

5.5.7.3. Reference Toxicants

Reference toxicants or positive controls are used to ensure that the organism on which the toxicity test is based is responding to a known contaminant in a reproducible way. This is particularly important for field-collected organisms, which may vary in response to a toxicant depending on season, collection site, temperature and handling.

Reference toxicants are also used to track changes in sensitivity of laboratory-reared or cultured organisms over time. Usually, either inorganic (e.g. copper, chromium) or organic (e.g. phenol, sodium dodecyl sulfate) reference toxicants are used and tested at a range of concentrations on a regular basis. In addition, each toxicity test should include at least one concentration of reference toxicant as a positive control. Quality control charts are produced showing the mean response and variability over time. Further guidance on the use of reference toxicant tests can be obtained from Environment Canada (1990b) and USEPA (1993).

5.5.7.4. Blanks

Appropriate field and process blanks should be included in each toxicity test if the sample has been manipulated before testing (see also section 4.6.3.1 in Chapter 4). If freshwaters have to be salinity adjusted with artificial sea salts before testing, sea salt controls should also be included in each test. Solvent controls are also essential for water insoluble chemicals if they have to be dissolved in organic solvents to deliver them into the test system. It is important to test a solvent control with the same concentration of solvent in clean water as is found in the highest test concentration. In no case should the concentration of solvents or emulsifiers exceed 0.1 mg/L (OECD 1981).

5.5.7.5. Quality of Ambient Water

Throughout the toxicity tests, the quality of the organisms' ambient water must be monitored to ensure that the toxicity measured is due to the contaminant or test sample alone, and to provide information that can be used in test interpretation. For freshwaters, measurements of alkalinity, hardness, pH, temperature and dissolved oxygen are the minimum parameters required. For marine studies, salinity is also monitored throughout the test.

5.5.8. QA/QC for Handling Sediments

The principles of handling sediment samples are similar to those for water analyses. Sample integrity must be maintained and QA/QC are important, as described in section 5.5.5. Some other aspects of the handling of sediment samples have been touched on already: the need to maintain unchanged redox conditions (oxic vs. anoxic) in the samples (section 4.3.5); and the need for negative controls and test samples to have similar particle sizes, organic carbon and sulfide contents (section 5.5.7.2). For more detailed advice on sediment handling and preparation, see Mudroch and Macknight (1991). Sediment sampling, handling and analysis are also described more fully in the Water Quality Guidelines, Volume 2, Appendix 8 (ANZECC & ARMCANZ 2000).

5.5.8.1 Pore Water Sampling

Pore waters in sediments can be sampled by pressure filtration or by centrifugation or by in situ methods such as pore water dialysis cells (peepers), gel samplers, or sippers. All operations should be conducted in an inert atmosphere. Filtration and centrifugation are the techniques most commonly used, but their suitability will depend on the sediment grain size. Solvent displacement has also been successfully applied in centrifugation methods.

The use of sippers or direct withdrawal techniques is limited to sandy sediments with a large pore volume. For investigation of pore water depth profiles, the peeper and gel sampling methods offer the best option.

5.5.8.2 Sample Storage

Sediment samples are typically either chilled or frozen immediately after collection, for storage and to minimise bacterial activity. Where total contaminant concentrations are of interest, either of these methods is suitable. In addition, oven drying at 110°C is also an option. For most organic compounds and the more volatile metals, e.g. Hg, Cd, Se, As, oven drying is unacceptable, and air drying at room temperature or freeze drying is preferable, although even these may be a concern with very volatile organic compounds.

Special considerations are required where metal speciation is of concern or where pore waters are to be analysed. Since most sediments are anoxic, at least in part, oxidation of iron sulfides in particular will change the chemical forms of metals in the pore water and solid sediment phases. This oxidation can be minimised by freezing the sample and storing it in a sealed container, and by preferably carrying out such operations in an inert atmosphere, e.g. under a nitrogen gas blanket or in a glove box. Freeze-drying can be done in an oxygen-free environment such as a glove box.

Freezing can rupture biological cells and release metals, and, although this is not generally an issue for sediments, it can significantly bias pore water results for some elements, e.g. selenium.

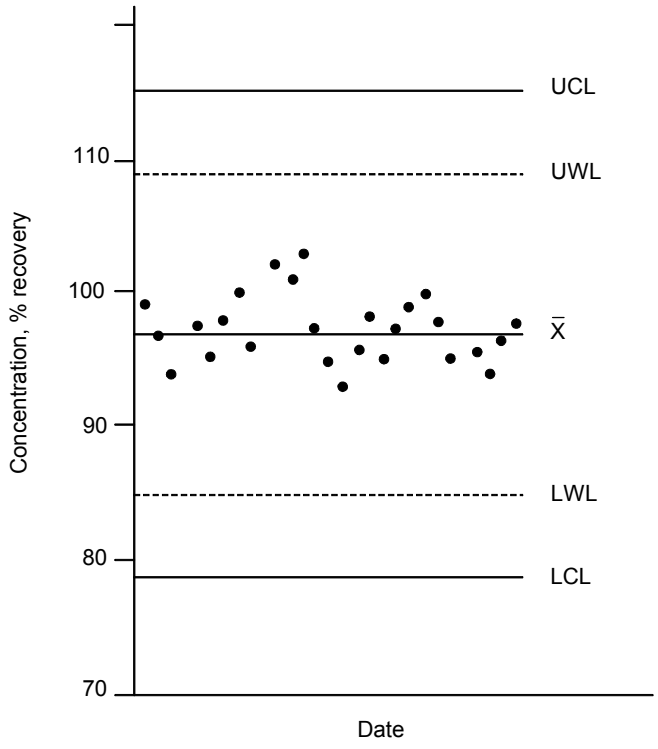

Figure 5.3. An example of a control chart for mean values; UCL = upper control limit; LCL = lower control limit; UWL = upper warning limit; LWL = lower warning limit

5.5.8.3. Sieving Samples

Sieving is the process used to divide sediment samples into fractions of different particle size. Sediments are usually classified as gravel (>2 mm), sand (63 µm to <2 mm), silt and clay (<63 µm). Sediments are usually sieved through a series of mesh sizes from 2 mm to 63 µm. Wet sieving is used for processing fine grain sediments while sieving of dry material is used for the separation of coarser material. When comparing trace metal concentrations in sediments from different sampling sites, it is normal to analyse the <63 µm fraction because it is this fraction that adsorbs most of the trace metals.

It should be noted that if organic contaminant concentrations in sediments are to be compared, grain size is not important but concentrations should be expressed as a percentage of organic carbon content.

5.5.8.4. Homogenisation of Samples

It is difficult to ensure the homogeneity of sediment samples being analysed because samples are notoriously heterogeneous with respect not only to particle size but also to contaminant distribution. Thorough mixing of any wet or dried samples is required to improve homogeneity. For dry samples, grinding with a mortar and pestle is necessary. Commercially available rock grinders are used to reduce larger dried particles to less than 63 µm for analysis. Coning and quartering, rolling, mechanical mixing and splitting are used for homogenisation and selection of material for analysis (Mudroch and MacKnight 1991).

Wet samples are used where it is feared that drying will alter the chemical form of the contaminants. For large wet sample volumes, homogenisation is especially difficult. It is more usual to use wet samples with smaller volumes, e.g. core sections. Here the wet samples can be homogenised by thorough mixing with a glass rod, then weighed out for analysis, with moisture determinations being carried out on separate aliquots of the sample.

5.5.9. Presentation of Quality Control Data

Control charts are used to visualise and monitor the variability in QC data (Lewis 1988). Two types of control charts are commonly used in laboratories: means charts and range charts.

- *Means charts* are used to track changes in certified reference concentrations, known additions, calibration check standards and blanks. The charts are graphs of the mean ± standard deviation or error over time (Figure 5.3) with a defined upper and lower control limit (normally three times the standard deviation where 99.7% of the data should lie). These are the limits. Data that fall above or below these limits are unacceptable and corrective action must be taken. Normally, action is taken if data are trending towards these limits.
- *Range charts* are used to track differences between duplicate analyses based on the standard deviation or relative standard deviation. Again, limits are set; when data fall above or below these limits, corrective action must be taken. For a full explanation of the procedures for calculating ranges refer to APHA (1998 or most current edition).

The number of quality control samples will depend on two considerations: the duration of acceptable deviations from the mean that can be tolerated, and the likelihood of deviations occurring. The first consideration will depend on the purpose for which the data are being collected, and the number of quality control samples will be set in consultation with the user of the data. The second consideration is usually based on past variability. To be effective, control charts must be continually updated as data become available so trends can be established before control limits are reached.

As a minimum, the precision and accuracy of data must be stated when data are presented in reports. Precision, as standard deviation or error, or relative standard deviation or error, should be presented graphically across the range of values being measured (Figure 5.4a). Alternatively, the standard deviation or similar should be given, at discrete values that cover the range of measurements. The method(s) of establishing the accuracy of the data should be stated. Bias should be presented graphically as a function of the true value over the applicable range (Figure 5.4b). Alternatively the bias at discrete values that cover the range of measurements should be given.

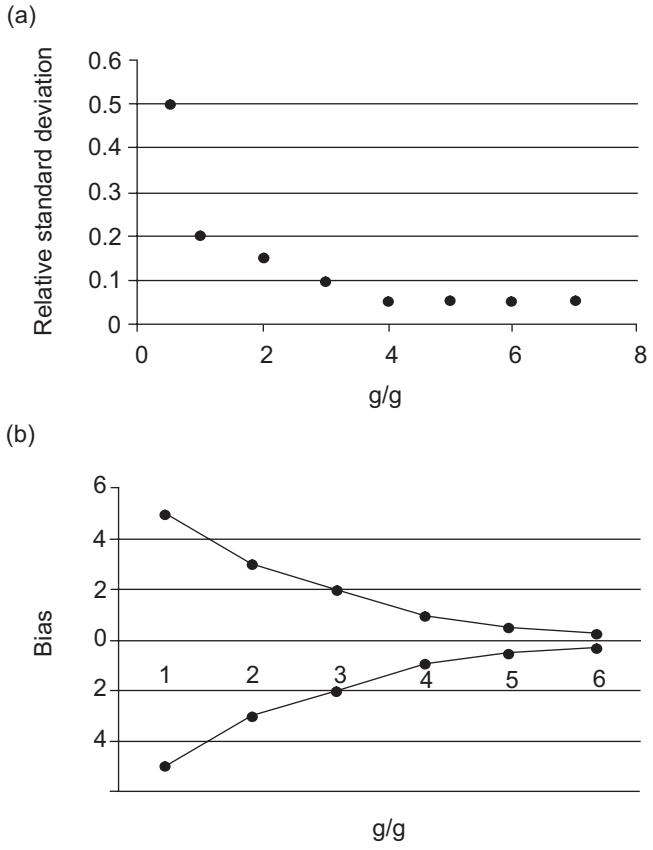

Figure 5.4. Graphical representation of (a) precision; (b) bias

5.6. Occupational Health and Safety

5.6.1. Legislative Requirements

Occupational health and safety requirements for laboratory work are provided in the various parts of Australian/New Zealand Standard AS/NZS 2243.2 (e.g. AS/NZS 1995, 1997a,b), *Safety in Laboratories*. This standard sets out the recommended procedures for safe working practices in laboratories.

Aspects covered include:

1. general;
2. chemical aspects;
3. microbiology;
4. ionizing radiators;
5. non-ionizing radiators;
6. mechanical aspects;
7. electrical aspects;
8. fume cupboards;
9. recirculating fume cupboards.

Practical guidance to the safety procedures and information needed for scientific work and safe laboratory practice is also available in *Safety Manual: An Essential Reference for Every Laboratory* (Haski et al. 1997).

5.6.2. Identification of Hazards

The hazards or risks involved with laboratory work need to be identified and documented. The major issues are:

- whether or not staff will be exposed to toxic or other hazardous substances;
- whether or not staff will be placed in a position of potential physical danger.

Staff who are to conduct analyses should be physically and mentally able to carry out laboratory work.

5.6.3. Education about Hazards

All staff must be appropriately trained as part of the formal risk minimisation strategy. Training will include:

- familiarisation with protocols (analysis procedures, safe handling procedures, disposal procedures, chain of custody considerations, etc.);
- use of laboratory equipment;
- qualifications in handling chemicals;
- familiarisation with safety procedures;
- qualifications in advanced first aid.

5.6.4. Risk Minimisation Plans

Proper professional practice requires that risks be reduced as much as possible, and that staff do not have to work in unsafe conditions.

Actions that can be taken to reduce risks include:

- the wearing of appropriate clothing and footwear to protect against accidental chemical spills;
- provision of an appropriate first aid kit in close proximity to where analyses are being undertaken;
- provision of an eye bath and safety shower in the laboratory;
- the training of laboratory staff in first aid procedures;
- staying in contact with help and never working alone; that is, at least three staff should work together and be in contact with someone who can raise an alarm. There should be written procedures describing how emergency services are to be contacted.