6 SELECTION AND APPLICATION OF AN ANALYTICAL METHOD

6.1 Introduction

This chapter provides guidance to both the project manager and the laboratory on the selection and application of analytical method. It offers guidance to the project manager on the development of the analytical protocol specifications (APSs) from the laboratory's perspective on method appropriateness and availability. It offers guidance to the laboratory on the key elements to consider when selecting an analytical method (Section 1.4.5, "Analytical Protocol") to meet the objectives of the APSs contained in the statement of work (SOW). Assuming that the laboratory has received a SOW, certain subsections within Section 6.5 provide guidance on how to review and properly evaluate the APSs therein. However, Section 6.5 also provides guidance for the project planning team on the important laboratory considerations needed to develop the measurement quality objectives (MQOs). Section 6.6 deals with method validation requirements and has been written for both the project planners and the laboratory.

Because the method constitutes the major part of the analytical protocol (Chapter 1), this chapter focuses on the selection of a method. However, other parts of the protocol should be evaluated for consistency with the method (Figure 6.1). MARLAP recommends the performance-based approach for method selection. Thus, the laboratory should be able to propose whichever method meets the project's analytical data requirements (MQOs), within constraints of other factors such as regulatory requirements, cost, and project deadlines. The selection of a method by the laboratory is in response to the APSs (Chapter 3) that were formulated during the directed planning process (Chapter 2) and documented in the SOW (Chapter 5, *Obtaining Laboratory Services*). In most project plan documents, the project manager or the project planning team has the authority and responsibility for approving the methods proposed by the laboratory. The APSs will, at a minimum, document the analytes, sample matrices, and the MQOs. A MQO is a statement of a performance objective or requirement for a particular method performance characteristic. The MQOs can be viewed as the analytical portion of the data quality objectives (DQOs; see Chapter 3).

Background material in Section 6.2.1 provides the reader with the subtleties of the performance-based approach to method selection, contrasted with the use of prescribed methods

Contents

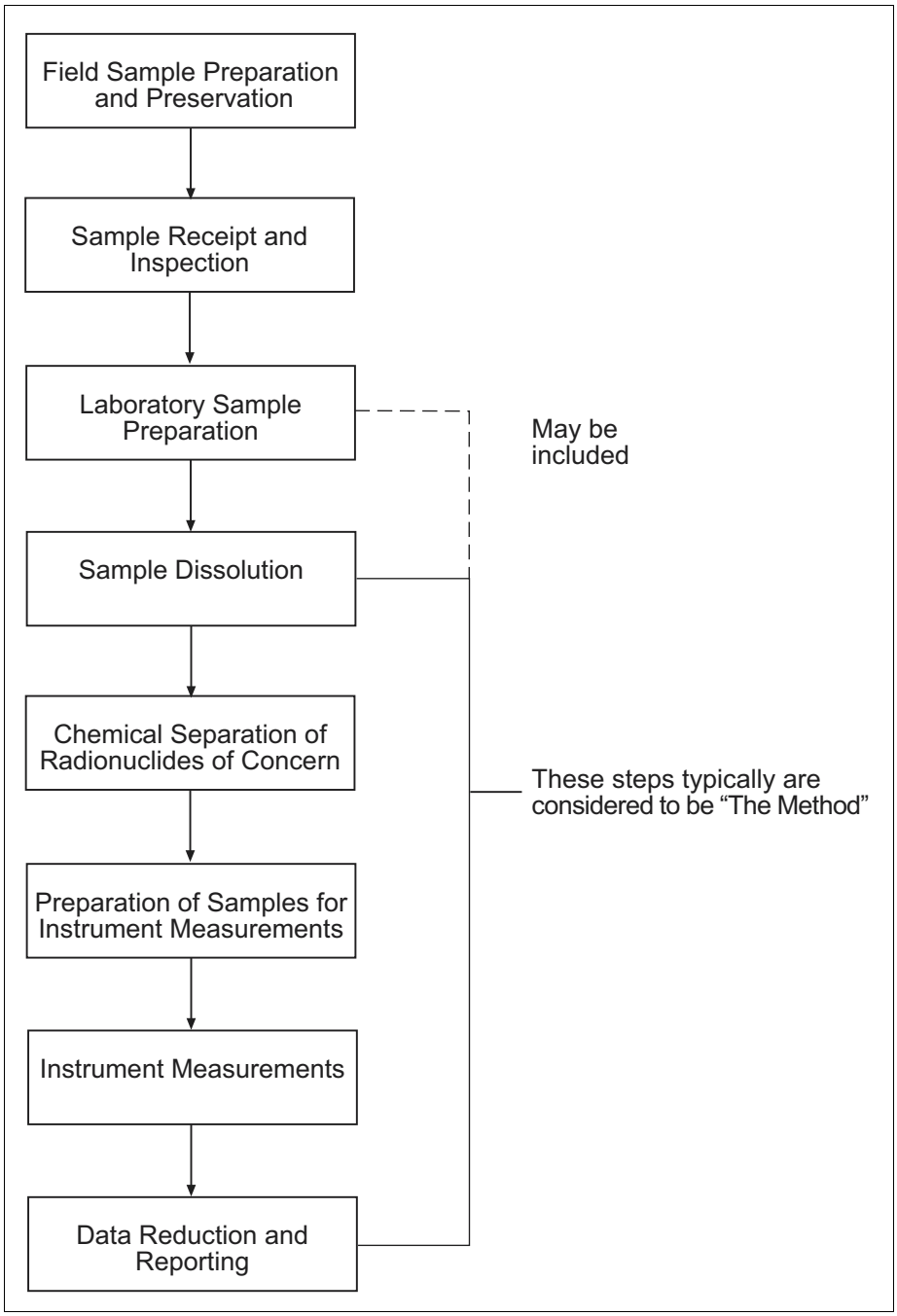

FIGURE 6.1 — Analytical process

and the importance of the directed planning process and MQOs in the selection of the method. This chapter does not provide a listing of existing methods with various attributes indexed to certain applications. Analytical methods may be obtained from national standards bodies, government laboratories and publications, and the open literature.

In this chapter, project method validation is defined as the demonstration of method applicability for a particular project. MARLAP recommends that only methods validated for a project's application be used. This recommendation should not be confused with the general method validation that all methods should undergo during method development. The laboratory should validate the method to the APS requirements of a SOW for the analyte/matrix combination and provide the project method validation documentation to the project manager prior to the implementation of routine sample processing (Section 6.6.2). If applicable, consideration should be given to the uncertainty of the laboratory's protocol for subsampling (heterogeneity) of the received field sample when selecting a method. Appendix F provides guidance on the minimization of subsampling uncertainty.

Section 6.3 provides an overview of the generic application of a method for a project and how a laboratory meets the recommendations of the guidance provided in this and other chapters. Generic considerations for the method selection process that a laboratory should evaluate are provided in Section 6.4. Project-specific considerations for method selection relevant to APSs are discussed in Section 6.5. Recommendations on the degree of project method validation specified by the project planning team are outlined in Section 6.6. Sections 6.7, 6.8, and 6.9 provide guidance on analyst qualifications, method control, and continued laboratory performance assessment, respectively. Section 6.10 outlines recommendations for the method proposal and method validation documentation that a laboratory should send to the project manager.

6.2 Method Definition

For this chapter, a laboratory "method" includes all physical, chemical, and radiometric processes conducted at a laboratory in order to provide an analytical result. These processes, depicted in Figure 6.1, may include sample preparation, dissolution, chemical separation, mounting for counting, nuclear instrumentation counting, and analytical calculations. This chapter will emphasize the laboratory's selection of the radioanalytical method that will be proposed in response to a SOW. Each method is assumed to address a particular analyte in a specified matrix or, in some cases, a group of analytes having the same decay emission category that can be identified through spectrometric means (e.g., gamma-ray spectrometry). However, it should be emphasized that the project planning team should have evaluated every component of the APSs for compatibility with respect to all analytes in a sample and the foreseen use of multiple analytical methods by the laboratory. For example, samples containing multiple analytes must be of sufficient size (volume or mass) to ensure proper analysis and to meet detection and quantification requirements. Multiple analytes in a sample will require multiple analyses for which a laboratory may use a sequential method that addresses multiple analytes or stand-alone individual methods for each analyte. The analytical protocol must ensure that the samples are properly preserved for each analyte and sufficient sample is collected in the field to accommodate the analytical requirements.

Certain aspects of a method are defined in this chapter in order to facilitate the method selection process. The following subsections describe the underlying basis of a performance-based approach to method selection and provide a functional definition related to MARLAP.

Performance-Based Approach and Prescriptive Method Application

MARLAP uses a performance-based approach to selecting a method, which is based on a demonstrated capability to meet defined project performance criteria (e.g., MQOs). With a properly implemented quality system, a validated method should produce appropriate and technically defensible results under the applicable conditions. The selection of any new method usually requires additional planning and, in some cases, may result in additional method development or validation. The selection of a method under the performance-based approach involves numerous technical, operational, quality, and economic considerations. However, the most important consideration in the selection of a method under the performance-based approach is compliance with the required MQOs for the analytical data. These requirements should be defined in the SOW or appropriate project plan document.

When developing the MQOs, the project planning team should have evaluated all processes that have a potential to affect the analytical data. Those involved in the directed planning process should understand and communicate the needs of the project. They should also understand how the sampling (field, process, system, etc.) and analytical activities will interact and the ramifications that the data may have on the decisionmaking process. These interactive analysis and communication techniques should be applied in all areas where analytical data are produced. As new projects are implemented, it should not be assumed that the current methods are necessarily the most appropriate and accurate; they should be reevaluated based on project objectives. The application of a performance-based approach to method selection requires the quantitative evaluation of all aspects of the analytical process. Once the MQOs for a project have been determined and incorporated into the APSs, under the performance-based approach, the laboratory will evaluate its existing methods and propose one or more methods that meet each APS. This chapter contains guidance on how to use the APSs in the laboratory's method evaluation process.

The objective of a performance-based approach to method selection is to facilitate the selection, modification, or development of a method that will reliably produce quality analytical data as defined by the MQOs. Under the performance-based approach, a laboratory, responding to a SOW, will propose a method that best satisfies the requirements of the MQO and the laboratory's operations.

In certain instances, the requirement to use prescribed methods may be included in the SOW. The term "prescribed methods" has been associated with those methods that have been selected by industry for internal use or selected by a regulatory agency, such as the U.S. Environmental Protection Agency (EPA), for specific programs. The methods for analyzing radionuclides in

drinking water prescribed by EPA (1980) provides an example of applying a limited number of methods to a well-defined matrix. In many companies or organizations, prescribed methods are widely used. Methods that have been validated for a specific application by national standard setting organizations such as the American Society for Testing and Materials (ASTM), American National Standards Institute (ANSI), American Public Health Association (APHA), etc., may also be used as prescribed methods by industry and government agencies.

Typically, the prescribed methods were selected by an organization to meet specific objectives for a regulation under consideration or for a program need. In most cases, the prescribed methods had undergone some degree of method validation, and the responsible organization had required a quality system to demonstrate continued applicability and quality, as well as laboratory proficiency. The use of any analytical method, whether prescribed or from the performance-based approach, has a life cycle that can be organized into the major categories of selection, validation, and continued demonstrated capability and applicability. This chapter will cover in detail only the first two of these categories. A discussion on ongoing laboratory evaluations is presented in Chapter 7 (*Evaluating Methods and Laboratories*) and Appendix C (*MQOs for Method Uncertainty and Detection and Quantification Capability*).

A final note should be made relative to prescribed methods and the performance-based approach to method selection. The performance-based approach for method selection allows more latitude in dealing with the potential diversity of matrices (such as waste-, sea-, ground- or surface water; biota; air filters; waste streams; swipes; soil; sediment; and sludge) from a variety of projects, or in dealing with different levels of data quality requirements or a laboratory's analytical proficiency. Even though the prescribed method approach may initially appear suitable and cost effective, it does not allow a laboratory to select a method from the many possible methods that will meet the MQOs.

Many individuals have the wrong impression that prescribed methods do not need to be validated by a laboratory. However, as discussed in this chapter, all methods should be validated to some level of performance for a particular project by the laboratory prior to their use. In addition, the laboratory should demonstrate continued proficiency in using the method through internal QC and external performance evaluation (PE) programs that use performance testing (PT) samples (Chapter 18, *Laboratory Quality Control*).

6.3 Life Cycle of Method Application

In responding to a SOW for a given analyte/matrix combination, a laboratory may have one or more methods that may be appropriate for meeting the MQOs. The final method selected from a set of methods may be influenced by many other technical, operational, or quality considerations. Figure 6.2 provides an overview of the life cycle of the method application. Figure 6.3 expands the life cycle into a series of flow diagrams.

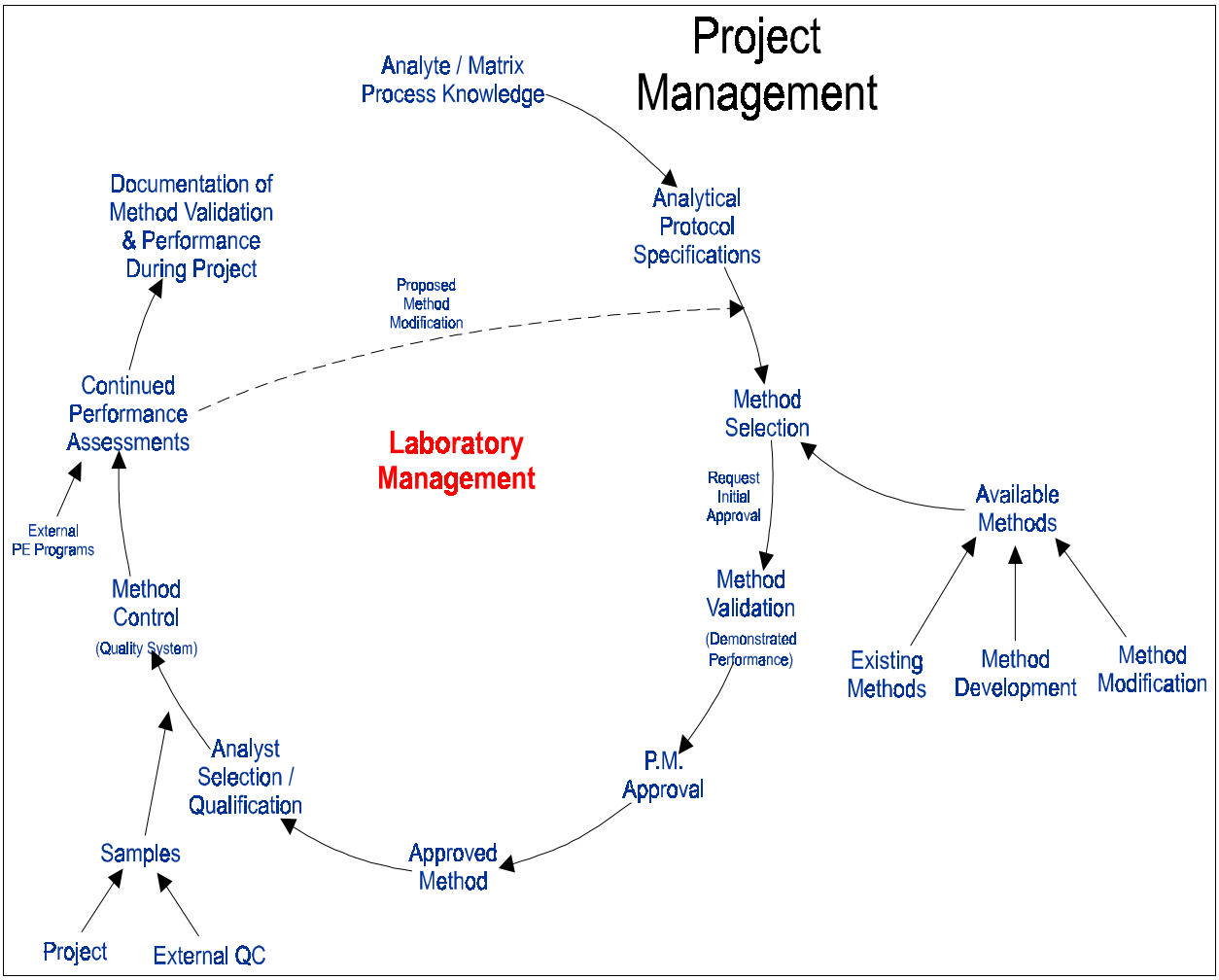

FIGURE 6.2 — Method application life cycle

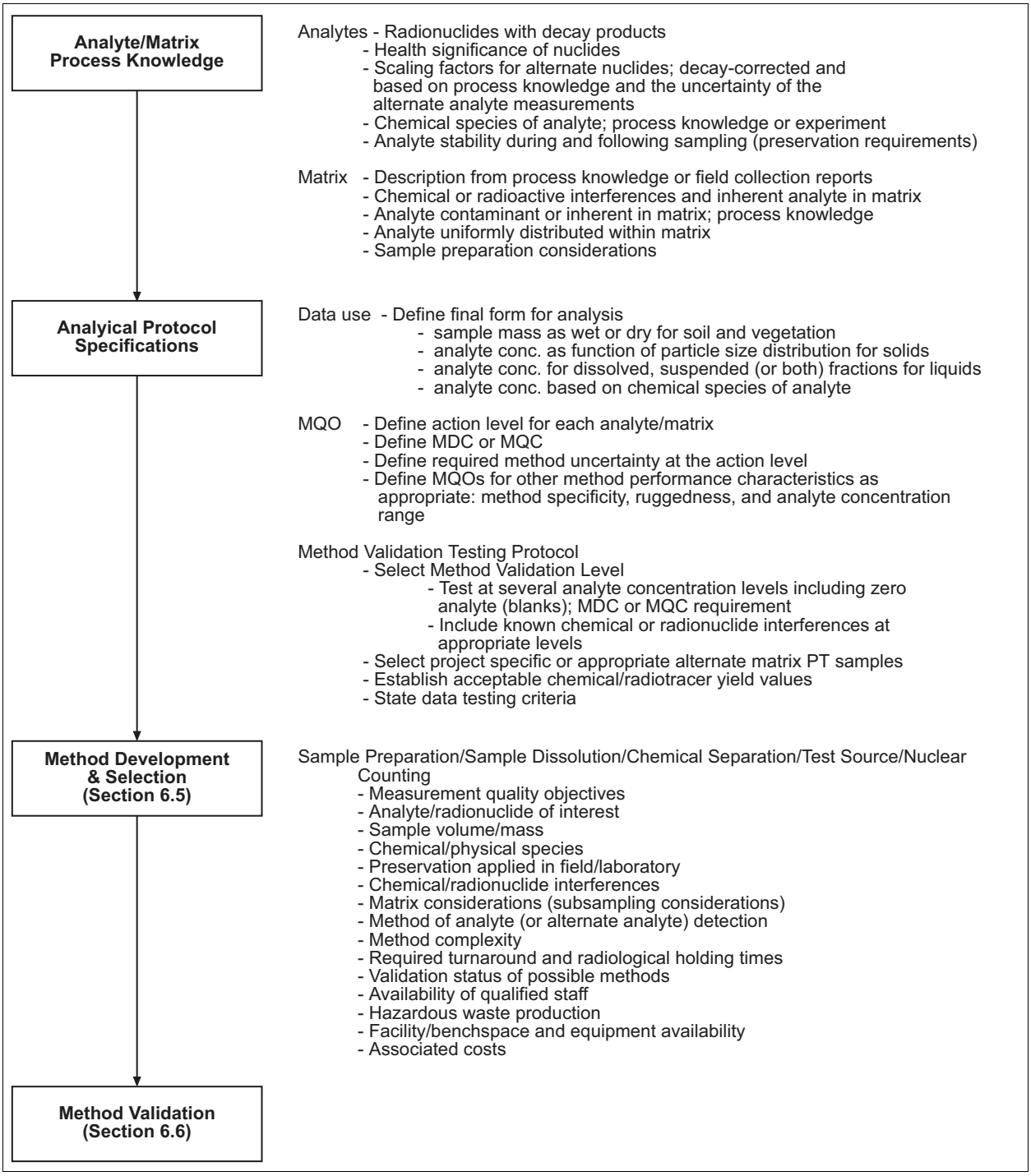

FIGURE 6.3 — Expanded Figure 6.2 addressing the laboratory's method evaluation process

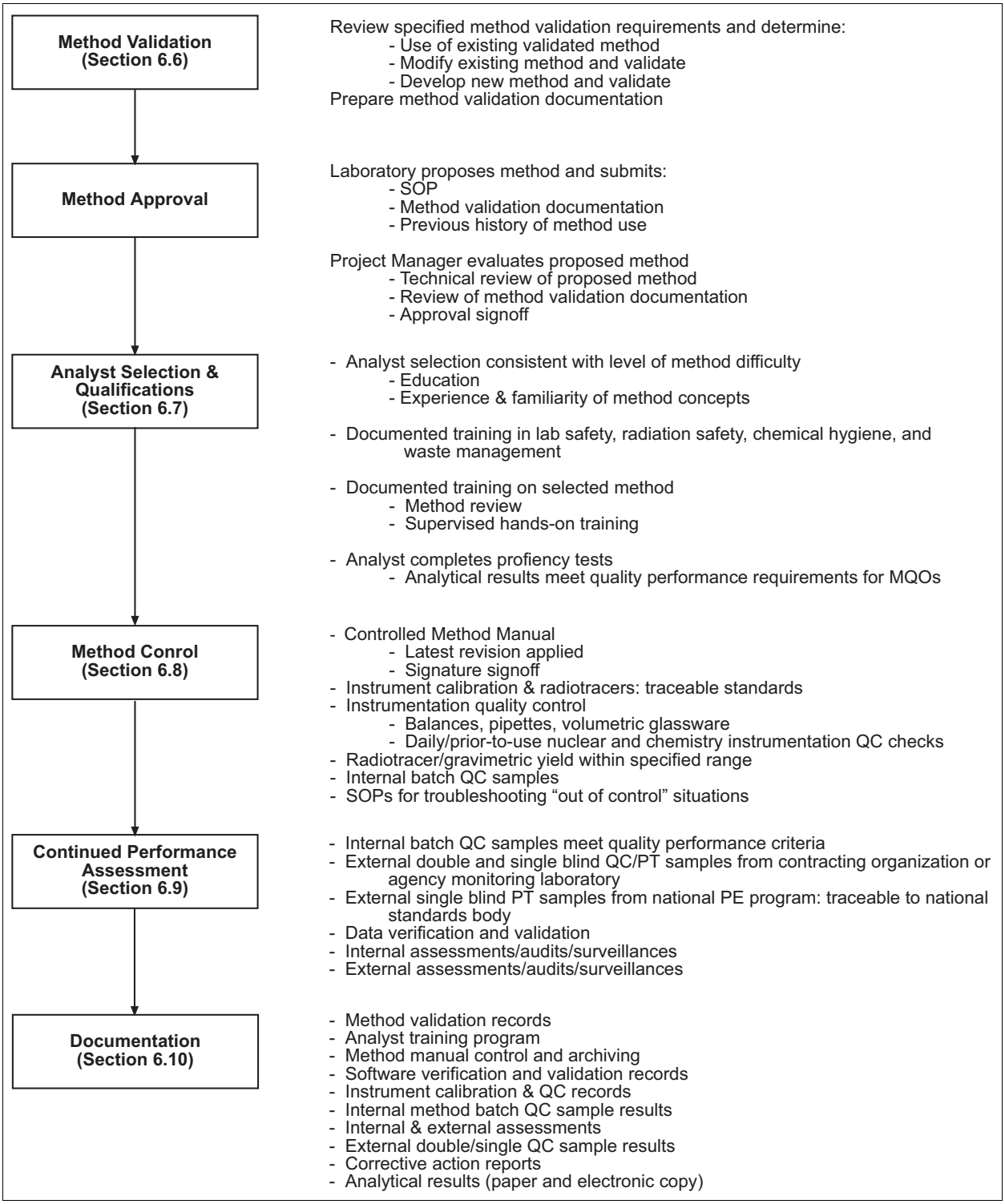

FIGURE 6.3 (cont’d) — Expanded Fig. 6.2 addressing the laboratory’s method evaluation process

6.4 Generic Considerations for Method Development and Selection

This section provides guidance on the technical, quality, and operational considerations for the development of a new method or the selection of an existing radioanalytical method. Unless required by a regulatory or internal policy, rarely should a method be specified in an APS or a SOW. MARLAP recommends that a SOW containing the MQOs and analytical process requirements be provided to the laboratory.

If the nature of the samples and analytes are known in advance, and variations in a sample matrix and analyte concentration are within a relatively small range, the development or selection of analytical methods is easier. In most situations, however, the number of samples, sample matrices, analyte interferences, chemical form of analytes, and variations among and within samples may influence the selection of a method for a given analyte. A number of radioanalytical methods are available, but no single method provides a general solution (all have advantages and disadvantages). The method selection process should consider not only the classical radiochemical methods involving decay emission detection (alpha, beta or gamma) but also non-nuclear methods, such as mass spectrometric and kinetic phosphorescence analysis.

In the performance-based approach to method selection, the laboratory may select and propose a gross measurement (alpha, beta, or gamma) method that can be applied to analyte concentrations well below the action level for the analyte, as well as an analyte specific method for analyte levels exceeding a proposed "screening level" that is a fraction of the action level. For example, it may be acceptable to propose a gross measurement method when its method uncertainty meets the method uncertainty (absolute or u_{MR}) requirement at concentration levels much below the action level. A gross measurement method may be employed initially for some projects. Such an approach would have to be agreed to by the laboratory and project manager. The project method validation, discussed in Section 6.6.2, should demonstrate that the gross measurement method can measure the analyte of interest (directly or indirectly) at a proposed analyte screening level concentration and meet the method uncertainty requirement (u_{MR}) in the presence of other radionuclides. Appendix C provides guidance on how to determine an acceptable method uncertainty at an analyte concentration relative to the action level.

In general, the development or selection of a method follows several broad considerations. These include analyte and matrix characteristics, technical complexity and practicality of methods, quality requirements, availability of equipment, facility and staff resources, regulatory concerns, and economic considerations. Each of the broad considerations can be detailed. The following list, although not inclusive, provides insight into the selection of an appropriate method. Many of these categories are discussed in subsequent MARLAP Part II chapters.

- Analyte/radionuclide/isotope of interest
  - Decay emission (particle or photon), atom detection, or chemical (photon detection)
  - Half-life of analyte

- Decay products (progeny); principal detection method or interference
- Chemical/physical forms (e.g., gas, volatile)
- Use of nondestructive or destructive sample analysis

- Level of other radionuclides or chemical interference
  - Level of decontamination or selectivity required, e.g., a decontamination factor of 10^3 for an interfering nuclide (^{60}Co) present with the analyte of interest (^{241}Pu)
  - Resolution of measurement technique
  - Ruggedness of technique for handling large fluctuations in interference levels and variations in a matrix
  - Radionuclides inherent in background

- Matrix
  - Destructive testing
    - Stable elemental interferences
    - Difficulty in dissolution of a matrix
    - Difficulty in ensuring homogeneity of aliquant
    - Inconsistency in chemical forms and oxidation states of the analyte versus the tracer
  - Non-destructive testing
    - Heterogeneity of final sample for analysis
    - Self absorption of particle/photon emissions within a matrix

- Degree of method complexity
  - Level of technical ability required of analysts
  - Reproducibility of quality results between analysts
  - Method applicability to sample batch processing
  - Extensive front-end chemical-processing technique (sample dissolution, analyte concentration and purification/isolation, preparation for final form for radiometrics)
  - Nuclear instrumentation oriented technique (minimal chemical processing)

- Required sample turnaround time
  - Half-life of analyte
  - Sample preparation or chemical method processing time
  - Nuclear instrumentation measurement/analysis time
  - Chemical or sample matrix preservation time
  - Batch processing
  - Degree of automation available/possible

- Status of possible methods and applications
  - Validated for the intended application
  - Staff qualified and trained to use method(s)
  - Existing method QC

- Specialized equipment, tracers, reagents, or materials available

- Hazardous or mixed-waste production
  - Older classical techniques versus new advanced chemical technologies
  - Availability and expense of waste disposal

- Associated costs
  - Labor, instrumentation usage, facilities, radiological waste costs
  - Method applicability to portable or mobile laboratory facilities
  - Availability of service hookups
  - Need for facility environmental controls
  - Need for regulatory permitting of mobile laboratory facility

6.5 Project-Specific Considerations for Method Selection

Certain parameters of the APSs (see Chapter 3 and the example in Figure 3.2) within the SOW are important to the method selection process. These include the analytes, matrix type, matrix characterization, analyte and matrix interferences, analyte speciation information gathered from process knowledge, sample process specifications (such as radiological holding times and sample processing turnaround times), and the MQOs. While these issues should be resolved during project planning, they are presented here as guidance to the laboratory for their review and evaluation of the technical adequacy of the SOW and to provide context for the method evaluation and selection process. Many of the issues from the project planning point of view are discussed in Section 3.3.

6.5.1 Matrix and Analyte Identification

The first step in selecting a method is knowing what analytes and sample matrices are involved. The following sections discuss what important information should accompany analyte and matrix identification.

6.5.1.1 Matrices

A detailed identification and description of the sample matrix are important aspects in the selection of an analytical method to meet the MQOs. The SOW should provide the necessary detailed sample matrix description, including those important matrix characteristics gathered from process knowledge. The laboratory should evaluate whether the existing sample preparation and dissolution steps of a method (Chapters 10 and 12 through 15) will be sufficient to meet the MQOs or the general or project method validation requirements. The matrix will also determine, to a certain extent, waste handling and disposal at the laboratory. If the matrix description is too vague or generic, the laboratory should contact the technical representative named in the SOW and request additional information.

The laboratory should ensure that the sample matrix description in the SOW reflects what is considered to be the "sample" by the project manager and the description is of sufficient detail to select the method preparation or analyte isolation steps that will meet the MQOs for the matrix. The laboratory should not accept generic sample matrix descriptions such as liquids or solids. For example, the differences between potable water and motor oil are obvious, but both may be described as a "liquid sample." However, there may be only subtle differences between potable surface water and groundwater but major differences between potable and process effluent waters. The laboratory should consider how much method ruggedness is needed in order to address the varied amounts of possible stable elements or compounds within a non-specified water matrix. Furthermore, when water from a standing pool is received in the laboratory, it may contain some insoluble matter. Now the questions arise whether the sample is the entire contents of the container, what remains in the container, the insoluble material, or just the water? A clay will act as an ion exchange substrate, while a sand may have entirely different retention properties. Both can be described as a soil or sediment, but the properties with which they retain a radionuclide are substantially different; thus, the method to properly isolate a particular radionuclide will vary. The laboratory should ensure that the selected method is consistent with the intended sample matrix, and the analytical results convey analyte concentration related to the proper matrix (i.e., Bq/L dissolved, Bq/L suspended, or Bq/L total). For such cases, the laboratory should request the project manager to clarify the "matrix" or "sample" definition.

Matrices generically identified as "solid" require additional clarification or information in order to select and validate a method properly. For example, sludges from a sewage treatment facility may be classified as a solid, but the suspended and aqueous portions (and possibly the dried residual material) of the sample may have to be analyzed. Normally, the radionuclide concentration in soils and sediments is reported in terms of becquerels per dry weight. However, certain projects may require additional sample process specifications (Section 6.5.4) related to the soil or sediment matrix identification that will affect the method selection process and the reporting of the data. This may involve sectioning of core samples, specified drying temperature of the sample, determining wet-to-dry weight ratio, removing organic material or detritus, homogenizing and pulverizing, sieving and sizing samples, etc. In order to determine the average analyte concentration of a sample of a given size containing radioactive particles, proper sample preparation and subsampling coupled with the applicable analytical methods are required (Chapter 12, *Laboratory Sample Preparation*, and Appendix F, *Laboratory Subsampling*). For alpha-emitting radionuclides, the method selected may only be suitable to analyze a few grams of soil or sediment, depending on the organic content. The laboratory should identify to the project manager the typical subsample or aliquant size that is used for the proposed method. Information should be provided to the laboratory on process knowledge. This information should indicate when sample inhomogeneities may exist due to:

- Radioactive particles;
- Selected analyte adsorption onto soil or sediment particles;
- Special chemical forms of the analyte; or

- Any other special analyte circumstances.

Based on this information, the laboratory should propose sample preparation and analytical methods that will address these matrix characteristics. Information on the solubility of the analyte can be used to select the dissolution method employed (see Chapter 13, *Sample Dissolution*). The laboratory should submit the proposed methods annotated with the suspected matrix characterization issues.

When selecting the methods for the analysis of flora (terrestrial vegetation, vegetables, aquatic plants, algae, etc.) or fauna (terrestrial or aquatic animals) samples, the detailed information on the matrix or the unique process specifications should be used by the laboratory to select or validate the method, or both. The laboratory should ensure that the specific units for the analytical results are consistent with the matrix identification and unique process specifications stated in the SOW. Most flora and fauna results are typically reported in concentrations of wet weight. However, for dosimetric pathway analyses, some projects may want only the edible portion of the sample processed and the results to reflect this portion, e.g., fillet of sport fish, meat and fluid of clams, etc. For the alpha- and beta-emitting radionuclides, aquatic vegetation normally is analyzed in the dry form, but the analyte concentration is reported as wet weight. The laboratory should ensure that the sample preparation method (Chapter 12) includes the determination of the necessary wet and dry weights.

These considerations bear not only on the method selected but also on how the sample should be collected and preserved during shipment. When possible, the laboratory should evaluate the proposed sample collection and preservation methods, as well as timeliness of shipping, for consistency with the available analytical methods. Discrepancies noted in the SOW for such collateral areas should be brought to the attention of the project manager. For example, sediment samples that have been cored to evaluate the radionuclide depth profile should have been collected and treated in a fashion to retain the depth profile. A common method is to freeze the core samples in the original plastic coring sleeves and ship the samples on ice. The SOW should define the specifics on how to treat the core samples and the method of sectioning the samples (e.g., cutting the cores into the desired lengths or flash heating the sleeves with subsequent sectioning).

The SOW should have properly delineated the proper matrix specifications required for project method validation. The purpose of the method validation reference material (MVRM) is to provide a matrix, which closely approximates that of the project samples to be analyzed (Section 6.6). The sample matrix must be characterized to the extent that the pertinent parameters are used to prepare the MVRM for the project method validation (Section 6.6.2). The laboratory should ensure that sufficient information and clarity have been provided on the matrix to conduct a proper method validation.

6.5.1.2. Analytes and Potential Interferences

The SOW should describe the analytes of interest and the presence of any other chemical and radionuclide contaminants (potential method interferences and their anticipated concentration) that may be in the samples. This information should be provided in the SOW to allow the laboratory's radiochemist to determine the specificity and ruggedness of a method that will address the multiple analytes and their interferences. The delineation of other possible interfering radionuclides is extremely important in the selection of a method to ensure that the necessary decontamination factors and purification steps are considered.

The size of the sample needed by the laboratory will depend on the number of analytes and whether the laboratory will select individual methods for each analyte or a possible "sequential" analytical method, where several analytes can be isolated from the same sample and analyzed. If a sample size is listed in the SOW, the laboratory should determine if there will be sufficient sample available to analyze all analytes, the associated QC samples, and any backup sample for re-analyses. Other aspects, such as the presence of short-lived analytes or analytes requiring very low detection limits, may complicate the determination of a proper sample size.

The laboratory should ensure that the project method validation requirements in the SOW are consistent with the analytes and matrix. The project method validation protocols defined in Section 6.6.2 are applicable to methods for single analyte analyses or to a "sequential method" where several analytes are isolated and analyzed. The laboratory should develop a well-planned protocol for project method validation that considers the method(s), analyte(s), matrix and validation criteria.

6.5.2 Process Knowledge

Process knowledge typically is related to facility effluent and environmental surveillance programs, facility decommissioning, and site remediation activities. Important process knowledge may be found in operational history or regulatory reports associated with these functions or activities. It is imperative that the laboratory review the information provided in the SOW to determine whether the anticipated analyte concentration and matrix are consistent with the scope of the laboratory operations. Process knowledge contained in the SOW should provide sufficient detail for the laboratory to determine, quickly and decisively, whether or not to pursue the work. If sufficient detail is not provided in the SOW, the laboratory should request the project planning documents. Laboratories having specialized sample preparation facilities that screen the samples upon arrival can make the necessary aliquanting or dilutions to permit the processing of all low-level samples in the laboratories. Laboratories that have targeted certain sectors of the nuclear industry or a particular nuclear facility may be very knowledgeable in the typical chemical and physical forms of the analytes of a given sample matrix and may not require detailed process knowledge information. However, under these circumstances, the laboratory's

method should be robust and rugged enough to handle the expected range of analyte concentrations, ratios of radionuclide and chemical interferences, and variations in the sample matrix.

Process knowledge may provide valuable information on the possible major matrix constituents, including major analytes, chemical/physical composition, hazardous components, radiation levels, and biological growth (e.g., bacteria, algae, plankton, etc.) activities. When provided, the laboratory should use this information to determine if the sample collection and preservation methodologies are consistent with the proposed radioanalytical method chosen. In addition, the information also should be reviewed to ensure that the proposed sample transportation or shipping protocols comply with regulations governing the laboratory operation.

Process knowledge information in the SOW may be used by the laboratory to refine method selection from possible radiometric/chemical interferences, chemical properties of the analytes or matrix, and hazardous components, among others. Chapter 14 describes the various generic chemical processes that may be used to ensure proper decontamination or isolation of the analyte from other interferences in the sample. These include ion exchange, co-precipitation, oxidation/reduction, and solvent extraction among others. The process knowledge information provided in the SOW should be reviewed to determine whether substantial amounts of a radionuclide that normally would be used as a radiotracer will be present in the sample. Similarly, information on the levels of any stable isotope of the analyte being evaluated is equally important. Substantial ambient or background amounts of either a stable isotope of the radionuclide or the radiotracer in the sample may produce elevated and false chemical yield factors. In addition, substantial amounts of a stable isotope of the analyte being evaluated may render certain purification techniques inadequate (e.g., ion exchange or solid extractants).

6.5.3 Radiological Holding and Turnaround Times

The SOW should contain the requirements for the analyte's radiological holding and sample turnaround times. MARLAP defines radiological holding time as the time differential between the date of sample collection and the date of analysis. It is important that the laboratory review the specifications for radionuclides that have short half-lives (less than 30 days), because the method proposed by the laboratory may depend on the required radiological holding time. For very short-lived radionuclides, such as ^{131}I or ^{224}Ra, it is very important to analyze the samples within the first two half-lives in order to meet the MQOs conveniently. A laboratory may have several methods for the analysis of an analyte, each having a different analyte detection and quantification capability. Of the possible methods available, the method(s) selected and proposed by the laboratory should address the time-related constraints of the radioanalytical process, such as the radiological holding time requirement, half-life of the analyte, and the time available after sample receipt at the laboratory. When a laboratory has several methods to address variations in these constraints, it is recommended that the laboratory propose more than one method with a clarification that addresses the radiological holding time and MQOs. In some cases, circumstances arise which require the classification of sample processing into several time-related

categories (Chapter 5). For example, the determination of ^{131}I in water can be achieved readily within a reasonable counting time through direct gamma-ray spectrometry (no chemistry) using a Marinelli beaker counting geometry, when the detection requirement is 0.4 Bq/L and the radiological holding time is short. However, when the anticipated radiological holding time is in the order of weeks, then a radiochemistry method using beta detection or beta-gamma coincidence counting would be more appropriate to meet the detection requirement. The more sensitive method also may be used when there is insufficient sample size or when the analyte has decayed to the point where the less sensitive method cannot meet the required MQOs. Another example would be the analysis of ^{226}Ra in soil, where the laboratory could determine the ^{226}Ra soil concentration through the quantification of a ^{226}Ra decay product by gamma-ray spectrometry after a certain ingrowth period, instead of direct counting of the alpha particle originating from the final radiochemical product (micro-precipitate) using alpha spectrometry.

Sample (processing) turnaround time normally means the time differential from the receipt of the sample at the laboratory to the reporting of the analytical results. As such, the laboratory should evaluate the SOW to ensure that the sample turnaround time, radiological holding time, data reduction and reporting times, and project needs for rapid data evaluation are consistent and reasonable. Method selection should take into consideration the time-related SOW requirements and operational aspects. When discrepancies are found in the SOW, the laboratory should communicate with the project manager and resolve any issue. Additionally, the response to the SOW should include any clarifications needed for sample turnaround time and/or radiological holding time issues.

6.5.4 Unique Process Specifications

Some projects may incorporate detailed sample processing parameters, specifications, or both within the SOW. Specifications for parameters related to sample preparation may include the degree of radionuclide heterogeneity in the final sample matrix prepared at the laboratory, the length of the sections of a soil or sediment core for processing, analysis of dry versus wet weight material, partitioning of meat and fluid of bivalves for analyses, and reporting of results for certain media as a dry or wet weight. Specifications related to method analysis could include radionuclide chemical speciation in the sample matrix. The laboratory must evaluate these specifications carefully, since various parameters may affect the method proposed by the laboratory. When necessary, the laboratory should request clarification of the specifications in order to determine a compatible method. In addition, the laboratory should ensure that the project method validation process is consistent with the unique process requirements. In some cases, not all special process specifications must be validated and, in other cases, site-specific materials (also referred to as MVRM) will be required for method validation. When necessary, the laboratory also should request site-specific reference materials having the matrix characteristics needed for proper method validation consistent with the special process requirements. It is incumbent upon the laboratory to understand clearly the intent of the special process specifications and how they will be addressed.

6.5.5 Measurement Quality Objectives

The specific method performance characteristics having a measurement quality objective may include:

- Method uncertainty at a specified analyte concentration level;
- Quantification capability (minimum quantifiable concentration);
- Detection capability (minimum detectable concentration);
- Applicable analyte concentration range;
- Method specificity; and
- Method ruggedness.

How each of these characteristics affect the method selection process will be discussed in detail in the subsequent paragraphs.

6.5.5.1 Method Uncertainty

From the directed planning process, the required method uncertainty at a stated analyte concentration should have been determined for each analyte/matrix combination. The method uncertainty requirement may be linked to the width of the gray region (Appendices B and C). MARLAP recommends that the SOW include the specifications for the action level and the required method uncertainty for the analyte concentration at the action level for each combination of analyte and matrix. For research and baseline monitoring programs, the action level and gray region concepts may not be applicable. However, for these applications, the project manager should establish a concentration level of interest and a required method uncertainty at that level. The laboratory should ensure that this method uncertainty requirement is clearly stated in the SOW.

The laboratory should select a method that will satisfy the method uncertainty requirement at the action level or other required analyte level. MARLAP uses the term "method uncertainty" to refer to the predicted uncertainty of a result that would be measured if a method were applied to a hypothetical laboratory sample with a specified analyte concentration. The uncertainty of each input quantity (method parameter) that may contribute significantly to the total uncertainty should be evaluated. For some methods, the uncertainty of an input quantity may vary by analyst or spectral unfolding software. Chapter 19 provides guidance on how to calculate the combined standard uncertainty of the analyte concentration, and Section 19.6.12 shows how to predict the uncertainty for a hypothetical measurement. For most basic methods, uncertainty values for the following input quantities (parameters) may be necessary when assessing the total uncertainty:

- Counting statistics (net count rate);
- Detector efficiency, if applicable;
- Chemical yield (when applicable) or tracer yield;

- Sample volume/weight;
- Decay/ingrowth factor; and
- Radiometric interference correction factor.

Typically, for low-level environmental remediation or surveillance activities, only those input quantities having an uncertainty greater than one percent significantly contribute to the combined standard uncertainty. Other than the radiometric interference correction factor and counting uncertainties, most input quantity uncertainties normally do not vary as a function of analyte concentration. At analyte levels near or below the detection limit, the counting uncertainty may dominate the method's uncertainty. However, at the action level or above, the counting uncertainty may not dominate.

When appropriate, the laboratory should determine the method uncertainty over the MQO analyte concentration range (Section 6.5.5.4), including the action level or other specified analyte concentration. The laboratory's project method validation (Section 6.6.2) should demonstrate or show through extrapolation or inference (e.g., from a lower or higher range of concentrations) that this method uncertainty requirement can be met at the action level or specified analyte concentration value. Method validation documentation should be provided in the response to the SOW.

6.5.5.2 Quantification Capability

For certain projects or programs, the project planning team may develop an MQO for the quantification capability of a method. The quantification capability, expressed as the minimum quantifiable concentration (MQC), is the smallest concentration of the analyte that ensures a result whose relative standard deviation is not greater than a specified value, usually 10 percent. Chapter 19 provides additional information on the minimum quantifiable concentration.

For example, if the MQC requirement for ^{89}Sr is 1.0 Bq/g (with a 10 percent relative standard deviation), the laboratory should select a method that has sufficient chemical yield, beta detection efficiency, low background, and sample (processing) turnaround time for a given sample mass to achieve a nominal measurement uncertainty of 0.1 Bq/g. The same forethought that a laboratory gives to estimating a method's minimum detectable concentration (MDC) for an analyte should be given to the MQC requirement. The laboratory should consider the uncertainties of all input quantities (detector efficiency, chemical yields, interferences, etc.), including the counting uncertainty when selecting a method. This is an important consideration, because for some methods, the counting uncertainty at the MQC level may contribute only 50 percent of the combined standard uncertainty. Therefore, the laboratory may have to select a method that will meet the MQC requirement for a variety of circumstances, including variations in matrix constituents and chemical yields, radionuclide and chemical interferences, and radioactive decay. In addition, sufficient sample size for processing may be critical to achieving the MQC specification.

During the project method validation process, the ability of the method to meet the required MQC specification should be tested. The method validation acceptance criteria presented in Section 6.6 have been formulated to evaluate the MQC requirement at the proper analyte concentration level, i.e., action level or other specified analyte concentration.

Since the laboratory is to report the analyte concentration value and its measurement uncertainty for each sample, the project manager or data validator easily can evaluate the reported data to determine compliance with the MQC requirement. Some projects may send PT material spiked at the MQC level as a more in-depth verification of the compliance with this requirement.

6.5.5.3 Detection Capability

For certain projects or programs, the method selected and proposed by the laboratory should be capable of meeting a required MDC for the analyte/matrix combination for each sample analyzed. For certain monitoring or research projects, the required analyte MDC may be the most important MQO to be specified in the SOW. For such projects, the MDC specification may be based on the analyte concentration of interest or the state-of-the-art capability of the employed technology or method. No matter what premise is used to set the value by the project planning team, the definition of, or the equation used to calculate, the analyte MDC should be provided in the SOW (Chapter 20). Furthermore, the SOW should specify how to treat appropriate blanks or the detector background when calculating the MDC. The laboratory should be aware that not all agencies or organizations define or calculate the MDC in the same manner. It is important for the laboratory to check that the SOW clearly defines the analyte detection requirements. In most cases, it would be prudent for the laboratory to use a method that has a lower analyte MDC than the SOW required MDC.

In some situations, a radiochemical method may not be robust or specific enough to address interferences from other radionuclides in the sample. The interferences may come from the incomplete isolation of the analyte of interest resulting in the detection of the decay emissions from these interfering nuclides. These interferences would increase the background of the measurement for the analyte of interest and, thus, increase the uncertainty of the measurement background. Consequently, an *a priori* MDC that is calculated without prior sample knowledge or inclusion of the interference uncertainties would underestimate the actual detection limit for the sample under analysis. Another example of such interferences or increase in an analyte's background uncertainty can be cited when using gamma-ray spectrometry to determine ^{144}Ce in the presence of ^{137}Cs. The gamma energy usually associated with the identification and quantification of ^{144}Ce is 133.5 keV. The gamma energy for ^{137}Cs is 661.6 keV. If a high concentration of ^{137}Cs is present in the sample, the Compton scattering from the 661.6 keV into the 133.5 keV region may decrease the ability to detect ^{144}Ce by one to two orders of magnitude over an *a priori* calculation that uses a nominal non-sample specific background uncertainty. Another example can be cited for alpha-spectrometry and the determination of isotopic uranium. If some interfering metal is present in unexpected quantities and carries onto the final filter mount or electro-

deposited plate, a substantial decrease in the peak resolution may occur (resulting in an increased width of the alpha peak). Depending on the severity of the problem, there may be overlapping alpha peaks resulting in additional interference terms that should be incorporated into the MDC equation. In order to avoid subsequent analyte detection issues, it is important for the laboratory to inquire whether or not the project manager has considered all the constituents (analytes and interferences) present in the sample when specifying a detection limit for an analyte.

The laboratory should include documentation in the response to the SOW that the method proposed can meet the analyte's MDC requirements for the method parameters (e.g., sample size processed, chemical yield, detector efficiency, counting times, decay/ingrowth correction factors, etc.). When practicable, care should be given to ensure the blank or detector background uncertainty includes contributions from possible anthropogenic and natural radionuclide interferences. In addition, any proposed screening method should meet the detection limit requirement in the presence of other radionuclide interferences or natural background radioactivity. When appropriate or required, the laboratory should test the method's capability of meeting the required MDC using MVRMs that have analytes and interferences in the expected analyte concentration range. Upon request, the project manager should arrange to provide MVRMs to the laboratory.

6.5.5.4 Applicable Analyte Concentration Range

The SOW should state the action level for the analyte and the expected analyte concentration range. The proposed method should provide acceptable analytical results over the expected analyte concentration range for the project. Acceptable analytical results used in this context means consistent method precision (at a given analyte concentration) and without significant bias. The applicable analyte concentration range may be three or four orders of magnitude. However, most radioanalytical methods, with proper analyte isolation and interference-decontamination steps, will have a linear relationship between the analytical result and the analyte concentration. For certain environmental monitoring or research projects, the laboratory should ensure that there are no instrument or analytical blank background problems. If the background is not well-defined, there may be an inordinate number of false positive and false negative results.

In its response to the SOW, the laboratory should include method validation documentation that demonstrates the method's capability over the expected range. The laboratory's project method validation (Section 6.6) should demonstrate or show through extrapolation or inference (e.g., from a different range of concentrations) that the method is capable of meeting the analyte concentration range requirement.

6.5.5.5 Method Specificity

The proposed method should have the necessary specificity for the analyte/matrix combination. Method specificity refers to the method's capability, through the necessary decontamination or separation steps, to remove interferences or to isolate the analyte of interest from the sample over

the expected analyte concentration range. Method specificity is applicable to both stable and radioactive constituents inherent in the sample. Certain matrices, such as soil and sediments, typically require selective isolation of femtogram amounts of the analyte from milligrams to gram quantities of matrix material. In these circumstances, the method requires both specificity and ruggedness to handle variations in the sample constituents.

If other radionuclide interferences are known or expected to be present, the SOW should provide a list of the radionuclides and their expected concentration ranges. This information enables the laboratory to select and propose a method that has the necessary specificity to meet the MQOs. As an alternative, the project manager may specify in the SOW the degree of decontamination a method needs for the interferences present in the samples. If the laboratory is not provided this information, method specificity cannot be addressed properly. The laboratory should ensure that related information on the matrix characteristics, radiometric or chemical interferences, and chemical speciation is provided to properly select a method.

6.5.5.6 Method Ruggedness

Ruggedness is the ability of the method to provide accurate analytical results over a range of possible sample constituents, interferences, and analyte concentrations, as well as to tolerate subtle variations in the application of the method by various chemists (EPA, 2002; APHA, 1998). Ruggedness is somewhat qualitative (Chapter 7). Therefore, the desirable parameters of a rugged method are difficult to specify quantitatively. A ruggedness test usually is conducted by systematically altering the critical variables (or quantities) associated with the method and observing the magnitude of the associated changes in the analytical results. ASTM E1169 provides generic guidance on how to conduct method ruggedness tests under short-term, high-precision conditions. In many cases, a rugged method may be developed over time (typically when difficulty is experienced applying an existing method to variations in the sample matrix or when two analysts have difficulty achieving the same level of analytical quality).

A laboratory may have several methods for an analyte/matrix combination. Samples from different geographical locations or different processes may have completely different characteristics. Therefore, the laboratory should select a method that is rugged enough to meet the APSs in the SOW. As indicated in Section 6.6.2, the prospective client may send site-specific MVRM samples for the method validation process or for PT samples (Chapter7).

6.5.5.7 Bias Considerations

As discussed earlier, the proposed method should provide acceptable analytical results over the expected analyte concentration range for the project. Acceptable results used in this context means consistent method precision (at a given analyte concentration) and without significant bias. According to ASTM (E177, E1488, D2777, D4855), “bias of a measurement process is a generic concept related to a constant or systematic difference between a set of test results from

the process and an accepted reference value of the property being measured," or "the difference between a population mean of the measurements or test results and the accepted reference or true value." ASTM (D2777) defines precision as "the degree of agreement of repeated measurements of the same property, expressed in terms of dispersion of test results (measurements) about the arithmetical mean result obtained by repetitive testing of a homogeneous sample under specified conditions." MARLAP considers bias to be a persistent difference of the measured result from the true value of the quantity being measured, which does not vary if the measurement is repeated. Normally, bias cannot be determined from a single result or a few results (unless the bias is large) because of the analytical uncertainty component in the measurement. Bias may be expressed as the percent deviation from a "known" analyte concentration. Note that the estimated bias, like any estimated value, has an uncertainty—it is not known exactly.

If bias is detected in the method validation process (see Section 6.6.4, "Testing for Bias") or from other QA processes, the laboratory should make every effort to eliminate it when practical. Implicitly, bias should be corrected before using the method for routine sample processing. However, in some cases, the bias may be very small and not affect the overall data quality. The project manager should review the method validation documentation and results from internal QC and external PE programs obtained during the laboratory review process (Chapter 7) and determine if there is a bias and its possible impact on data usability.

6.6 Method Validation

Without reliable analytical methods, all the efforts of the project may be jeopardized. Financial resources, timeliness, and public perception and confidence are at risk, should the data later be called into question. Proof that the method used is applicable to the analyte and sample matrix of concern is paramount for defensibility. The project manager should ensure the methods used in the analyses of the material are technically sound and legally defensible.

The method selected and proposed by the laboratory must be based on sound scientific principles and must be demonstrated to produce repeatable results under a variety of sample variations. Each step of the method should have been evaluated and tested by a qualified expert (radio-analytical specialist) in order to understand the limits of each step and the overall method in terms of the MQOs. These steps may involve well-known and characterized sample digestion, analyte purification and decontamination steps that use ion exchange, solvent extraction, precipitation and/or oxidation /reduction applications. Method validation will independently test the scientific basis of the method selected for a given analyte and sample matrix.

EURACHEM (1998) interprets method validation as "being the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. Implicit in this is that it will be necessary to evaluate the method's performance capabilities." As such, the laboratory is responsible for

ensuring that a method is validated adequately. MARLAP distinguishes between general method validation and project method validation. During the development of an analytical method or prior to the first use of a recognized industry or government method, laboratories typically perform a general method validation. General method validation is normally conducted to determine the capability of the method for a single analyte/matrix combination to meet internal laboratory quality requirements.

For the purposes of MARLAP, project method validation is the demonstration that the radioanalytical method selected by the laboratory for the analysis of a particular radionuclide in a given matrix is capable of providing analytical results that meet a project's MQOs and any other requirements in the APS. A proposed method for a specific combination of analyte and matrix should be validated in response to the requirements within a SOW. Demonstration of method performance to meet project-specific MQOs prior to analyzing project samples is a critical part of the MARLAP process.

Methods obtained from recognized industry standards (ASTM, ANSI, APHA) or government method manuals may have been validated for certain general applications by the developing or issuing laboratory. However, prior to their use, other laboratories planning to use these methods need to perform general and project method validations to ensure that the method meets laboratory performance criteria (for generic applications) and project method validation criteria, respectively. In some cases, the laboratory's quality requirements and method attributes for general method validation may be less stringent compared to a project method validation. For example, a method's precision or chemical yield range requirement may be less stringent for general method validation than for project method validation requirements. MARLAP recommends that a method undergo some basic general validation prior to project method validation.

In the discussion on general and project method validation, certain terms related to test samples are used. These include method validation reference materials (MVRMs) and internal and external PT materials. MVRM refers to site-specific materials that have the same or similar chemical, physical, and nuclear properties as the proposed project samples. Normally, MVRMs can be prepared by at least two mechanisms:

- Spiking background or blank material from a site with the radionuclides of interest; or
- Characterizing the site material containing the radionuclides of interest to a high degree of accuracy.

Although MVRM is the most appropriate material for testing a laboratory's project-specific performance or for validating a method for a particular project, its availability may be limited depending on the project manager's ability to supply such material. Internal PT materials (samples) are materials prepared by the laboratory, typically as part of a laboratory's QC program and method validation process. A matrix spike (internal batch QC sample) may be considered an

internal PT material. External PT materials are materials prepared for use in an external government or commercial PE program. When available and applicable, external PT samples may be used for validating methods. PT and MVRM samples should be traceable to a national standards body, such as the National Institute of Standards and Technology in the United States.

An analytical laboratory's quality system should address the requirements and attributes for general method development, including some level of validation. However, general validation will not address the specific requirements of project method validation. MARLAP recommends that when a method is applied to a specific project, the method should then undergo validation for that specific application.

6.6.1 General Method Validation

A general method validation process should be a basic element in a laboratory's quality system. General method validation is applied to an analyte(s)/matrix combination, such as ^{90}Sr in water, but can be applied to a "sequential" method to determine multiple analytes. In most cases, a matrix of typical constituents will be used when evaluating the method. A general method validation protocol should address the important aspects of the methods that influence the results (e.g., inclusion of radiotracers, standard addition, alternate analyte analyses, etc.) and the basic quality requirements of a laboratory's quality system. General guidance on single laboratory method validation can be found in IUPAC (2002) and EURACHEM (1998). For most applications, the method should be evaluated for precision and relative bias for several analyte concentration levels. In addition, the absolute bias, critical level and the *a priori* minimum detectable concentration of the method, as determined from appropriate blanks, should be estimated. (See Section 6.6.4 for a discussion on testing for absolute and relative bias.) There should be a sufficient number of test level concentrations and replicate PT samples to make realistic estimates of the quality parameters. During validation, the method should also be evaluated in terms of factors most likely to influence a result (e.g., ruggedness) so that the method can handle minor deviations to the method and precautions may be written into the method (Youden and Steiner, 1975). In addition, IUPAC (2002) recommends that method validation evaluate the following parameters: applicability, selectivity, calibration and linearity, range of applicable analyte concentrations, detection and determination (quantification capability) limit, sensitivity, fitness of purpose, matrix variation and measurement uncertainty.

Laboratories that have participated in an interlaboratory collaborative study whose data were included in a published method (having an appropriate number of test levels and replicate samples, e.g., ASTM D2777 and Youden and Steiner, 1975) would be considered to have an acceptable general validated method for the analyte/matrix combination under study. These collaborative studies have at a minimum three or four different analyte concentration levels (excluding blanks) with three replicates or Youden pairs per analyte concentration level. A well-planned collaborative study will include expected interferences and matrix variations.

6.6.2 Project Method Validation Protocol

A laboratory's project method validation protocol should include the evaluation of the method for project-specific MQOs for an analyte and internal quality performance criteria as well as other generic parameters. With a properly designed method validation protocol, important information may be ascertained from the analytical results generated by the method validation process.

The parameters that should be specified, evaluated, or may be ascertained from the analytical results generated by the project method validation process are listed below:

- Defined Method Validation Level (Table 6.1)
- APSs including MQOs for each analyte/matrix
  - Chemical or physical characteristics of analyte when appropriate
  - Action level (if applicable)
  - Method uncertainty at a specific concentration
  - MDC or MQC
  - Bias (if applicable)
  - Applicable analyte concentration range
  - Method blanks
  - Other qualitative parameters to measure the degree of method ruggedness or specificity
- Defined matrix for testing, including chemical and physical characteristics that approximate project samples
- Selected project-specific or appropriate alternative matrix PT samples, including known chemical or radionuclide interferences at appropriate levels
- Defined sample preservation
- Stated additional data testing criteria (such as acceptable chemical/radiotracer yield values)

In order to demonstrate properly that a method will meet project MQOs, the method should be evaluated over a range of analyte concentrations that cover the expected analyte concentration range for the project (Section 6.5.5.4). The middle of the concentration range should be set near the action level. The preparation and analysis of the test samples should result in a measurement uncertainty that is equal to or less than the required method uncertainty. In addition, anticipated or known chemical and radionuclide interferences should be added in the appropriate "interference to analyte" activity or concentration ratio. As a requirement of the project method validation process, appropriate method blanks (containing similar interferences when practical) should be analyzed concurrently with the matrix spikes to determine analyte interferences and to estimate the absolute bias near the detection limit (Section 6.6.4, "Testing for Bias").

The number of validation samples requires is a function of the validation level sought. As shown in Table 6.1, the number of samples may vary from 9 to 21.

TABLE 6.1 — Tiered project method validation approach

Validation Level	Application	Sample Type*	Acceptance Criteria§	Levels † (Concentrations)	Replicates	No. of Analyses
A Without Additional Validation	Existing Validated Method	—	Method Previously Validated (By One of the Validation Levels B through E)	—	—	—
B	Same or Similar Matrix	Internal PT	Measured Value Within $\pm 2.8u_{MR}$ or $\pm 2.8\varphi_{MR}$ of Known Value	3	3	9
C	Similar Matrix/New Application	Internal or External PT	Measured Value Within ± 2.9 u_{MR} or $\pm 2.9\varphi_{MR}$ of Known Value	3	5	15
D	Newly Developed or Adapted Method	Internal or External PT	Measured Value Within ± 3.0 u_{MR} or $\pm 3.0\varphi_{MR}$ of Known Value	3	7	21
E	Newly Developed or Adapted Method	MVRM Samples	Measured Value Within ± 3.0 u_{MR} or $\pm 3.0\varphi_{MR}$ of Known Value	3	7	21

* PT and MVRM samples should be traceable to a national standards body, such as NIST in the United States. Internal PT samples are prepared by the laboratory. External PT samples may be obtained from a performance evaluation program or from a commercial radioactive source producer that has traceability to a national standards body. Blank samples should be representative of the matrix type being validated.

§ The acceptance criterion is applied to each analysis in the method validation, not to the mean of the analyses. u_{MR} is the required absolute method uncertainty for analyte concentrations at or below the action level and φ_{MR} is the required relative method uncertainty for analyte concentrations above the action level (see Figure C.1 in Appendix C). The acceptance criteria are chosen to give a false rejection rate of ~5% when the measurement process is unbiased, with a standard deviation equal to the required method uncertainty (u_{MR} or φ_{MR}). The stated multiplier, k, for the required method uncertainty was calculated using the formula $k = z_{0.5 + 0.5(1-\alpha)^{1/N}}$ where N is the number of measurements, α is the desired false rejection rate, and, for any p, z_p denotes the p-quantile ($0 < p < 1$) of the standard normal distribution.

† Concentration levels should cover the expected analyte concentration range for a project including the action level concentration. A set of five appropriate blanks (not considered a level) should be analyzed during the method validation process. The blank data and the estimated absolute bias in the mean blank concentration value (see Attachment 6A in this chapter for applicable statistical tests) shall be reported as part of the method validation documentation.

6.6.3 Tiered Approach to Project Method Validation

While MARLAP recommends that as each new project is implemented, the methods used in the analysis of the associated samples undergo some level of validation, it is the project manager's responsibility to assess the level of method validation necessary. Although the end result of method validation is to ensure that the selected method meets the MQOs for an analyte/matrix, the level of validation depends on the extent of method development. Therefore, MARLAP recommends a tiered approach for project method validation. The recommended level of validation for new or existing methods are provided in the next four sections, based on level of effort: no additional validation, modification of a method for a similar matrix, new application of

a method, and newly developed or adapted methods. The suggested levels of validation are indicative of the modification required of the method. It should be noted that the method validation requirements of Table 6.1 permit the laboratory to use internal and external PT or site-specific MVRM samples and also permit the project manager to provide PT or site-specific MVRM samples for the laboratory to use or analyze. As part of the qualifying process, a project manager may provide PT samples. In this case, the project manager should ensure consistency with the method validation requirements of Table 6.1. Most laboratories normally have documentation on the general or overall performance of a method. This documentation may supplement, or occasionally may be sufficient to meet the project method validation criteria.

The tiered approach to project method validation outlined in Table 6.1 was developed to give the project manager flexibility in the method validation process according to the project MQOs The degree of method validation increases from the lowest (Level A) to the highest (Level E). Figure 6.4 illustrates that—for a given validation level—the relative assurance in a method meeting the MQOs and the relative effort for method validation required by the laboratory are directly related. For certain projects, achieving the highest degree of assurance in method suitability (e.g., for a difficult sample matrix with interferences) would require validation using site-specific PT samples (Level E). This validation level also requires 21 samples: more laboratory effort compared to the other levels.

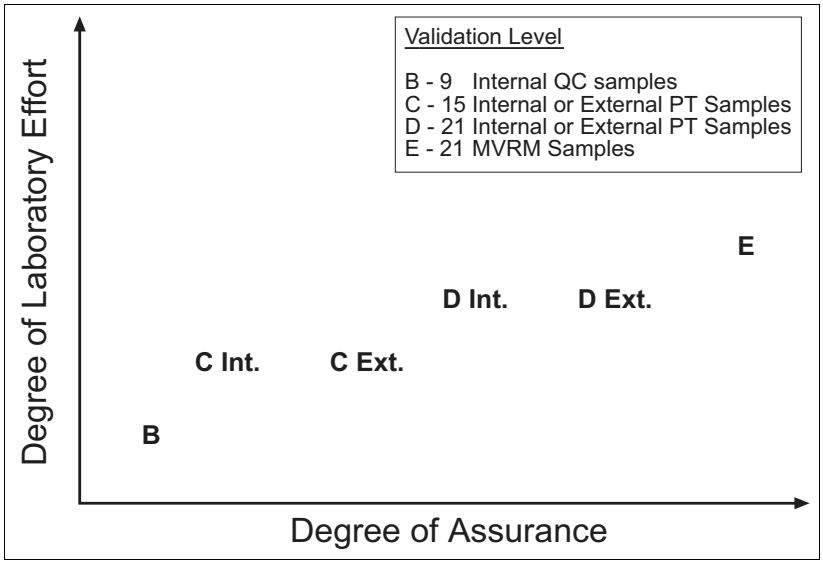

FIGURE 6.4 — Relationship between level of laboratory effort, method validation level, and degree of assurance of method performance under the tiered approach to method validation

Each of the validation levels evaluates the proposed method over the expected concentration range of the analytes and interferences. Requiring that each analytical result be within the interval of the known value ± ~3 times the required method uncertainty (u_{MR} or φ_{MR}) at the action level ensures a high degree of confidence that a method will meet the MQO. (See Appendix C for the

definition of the required method uncertainty at the action level or other stated concentration, u_{MR}.) In addition to evaluating the method uncertainty, the method should be evaluated for bias (Section 6.6.4).

During the method validation process, the laboratory should ensure that the standard deviation for the samples analyzed is consistent with the estimated individual sample measurement uncertainty. An evaluation should be conducted for replicate sample analyses that have the same approximate relative measurement uncertainties. If the estimated measurement uncertainty of a given sample is much different than the observed method precision for the replicate analyzes, then the laboratory may not have properly estimated the uncertainty of one of the input parameters used to calculate the combined standard uncertainty.

6.6.3.1 Existing Methods Requiring No Additional Validation

For completeness, it is necessary to consider the possibility that a previously validated method requires no additional validation (Level A of Table 6.1) for a specific project. As noted in the table, the method should have previously undergone some level (Level B through E) of validation. It may be that the samples (matrix and analyte specific) associated with a new project are sufficiently similar to past samples analyzed by the same laboratory that the project manager feels additional validation is unwarranted. The decision to use Level A method validation should be made with caution. While the sampling scheme may be a continuation, the analytical processing capabilities at the laboratory may have changed sufficiently to merit limited method validation. Without some level of method validation, the project manager has no assurance that the analytical laboratory will perform to the same standards as an extension of the earlier work.

6.6.3.2 Routine Methods Having No Project Method Validation

When a laboratory has a routine method for a specific radionuclide/matrix combination that has had no previous project method validation, a project manager may select method validation Level B to validate the method for project sample analyses. Since the routine method has been used on a regular basis for client and PE program samples, there should be sufficient information on the performance of the method. As such, the minimum method validation protocol of Level B should be adequate to verify the method's performance.

6.6.3.3 Use of a Validated Method for Similar Matrices

When a previously validated method is to be used in the analysis of samples that are similar to the matrix and analyte for which the method was developed, MARLAP recommends that validation of the method be implemented according to Level B or C of Table 6.1. These levels will provide a reasonable assurance to both the laboratory and the project manager that the method will meet the required MQOs. Level B requires the least amount of effort for the laboratory but may not satisfy the level of method validation required by the project. When the

laboratory does not have the capability to produce internal QC samples, the Level C validation protocol should be used.

Since a method inherently includes initial sample preparation, projects that have severe differences in analyte heterogeneity may require a moderate change in a radiochemical method's initial sample treatment. A change in the method to address the increased heterogeneity of the analyte distribution within the sample may require another method validation depending on the ruggedness of the method and the degree of analyte heterogeneity. In this case, Level C validation would be appropriate.

6.6.3.4 New Application of a Validated Method

Methods that have been validated for one application normally require another validation for a different application, such as a different sample matrix. In addition, the MQOs may change from one project to another or from one sample matrix to another. The validation process for an existing validated method should be reviewed to ensure applicability of the new (which can be more or less restrictive) MQOs. Applying an existing method to another matrix is not recommended without further method validation. MARLAP recommends, based on the extent of the modification and the difficulty of the matrix, that Level C of Table 6.1 be used to validate the performance of the modified method.

Both internal and external PT samples may be used for Level C validation. However, the project manager should specify the PT matrix. It should be recognized that national or commercial PE programs may not provide the necessary matrices or the required analyte concentrations needed for the Level C validation protocol. However, some radioactive source suppliers have the capability to produce high quality PT materials for method validation.

Validation of an existing method for a different application depends on the extent of the departure from the original method application, in terms of:

- Dissimilarity of matrices;
- Chemical speciation of the analyte or possible other chemical interference;
- Analyte, chemical or radiometric interferences;
- Complete solubilization of the analyte and sample matrix; and
- Degree of analyte or sample matrix heterogeneity.

When the chemical separation of the analyte varies from that for which the method was originally validated, the method should be so modified and subsequent validation performed. For example, if the original method was developed and validated to extract iodide using ion exchange chromatography, and a new application requires that iodine and iodate be quantified as well as iodide, then the method should be validated for the new analytes. Another example would be the initial development of a method for soluble plutonium in soil using acid dissolution and then

applying the same method to high-fired plutonium oxide in soil. For these two examples, if the original methods were to undergo the validation process for the new application, definite deficiencies and poor results would be evident. Portions of the original method would have to be modified to address the chemical speciation problems. The modified method requires validation to ensure that the MQOs for the new application can be met.

When additional analyte, chemical, or sample matrix interferences are known to exist for a new application, the previously validated method should undergo further validation. For example, applying a method developed for the analysis of an analyte in an environmental matrix containing few interfering radionuclides would be inappropriate for the analysis of process waste waters containing many interfering radionuclides at high concentrations. In essence, the degree of decontamination (degree of interference removal) or analyte purification (isolation of the analyte from other radionuclides) necessary for one application may be completely inadequate or inappropriate for another application (an indication of method specificity).

Another example would be the use of a method for soil analysis employing ^{234}Th as a radiotracer for chemical yield for the isotopic analysis of thorium when the soil also has a high concentration of uranium. Thorium-234 is a decay product of ^{238}U and will exist in the sample as a natural analyte, thus creating an erroneous chemical yield. A third example is the application of a ^{90}Sr method developed for freshwater to seawater samples for which the amount of chemical interferences and ambient strontium levels are extensive.

Some matrices and analytes may be solubilized easily through acid dissolution or digestion. For some applications, the analyte of interest may be solubilized from the sample matrix through an acid extraction process. The applicability of such methods should be carefully chosen and, most important, the method must be validated for each application. Definite problems and misapplication can be the result of using an acid extraction process when a more robust complete sample dissolution is necessary. These examples illustrate the deficiencies of the initial method validation when applied to the modified sample parameters.

6.6.3.5 Newly Developed or Adapted Methods

MARLAP recommends that methods developed by the laboratory or adapted from the literature that have not been previously validated for a project be validated according to Levels D or E of Table 6.1. These levels provide the most comprehensive testing of method performance. Levels D and E have an increased number of replicates and the data obtained should provide the best estimate of a method's precision and bias. When the matrix under consideration is unique, the method should be validated using the same matrix (e.g., MVRM) as determined in Level E. This is extremely important for process/effluent waters versus laboratory deionized water and for various heavy metal radionuclides in soils or sediments when compared to spiked sand or commercial topsoil. For site-specific materials containing severe chemical and radionuclides

interferences, many methods have been unable to properly address the magnitude of interferences.

6.6.4 Testing for Bias

The laboratory should test the method for bias.[1] In fact, the laboratory should check for at least two types of bias: *absolute* and *relative*. Attachment 6A describes a statistical hypothesis test that may be used to check for each type.

It is assumed here that the mean response of the method is an essentially linear function of analyte concentration over the range of the method. This function can be characterized by its *y*-intercept, which equals the mean response at zero concentration, and its slope, which equals the ratio of the change in the mean response to a change in sample analyte concentration. The absolute bias of the method is equated here with the y-intercept, and the relative bias is equated with the difference between the slope and 1.

Detecting and quantifying an absolute or relative bias in a measurement process may be difficult if the bias is small in relation to the uncertainty of a measurement. Typically, an absolute bias is most easily observed by analyzing blank samples, and a relative bias is most easily observed by analyzing high-activity certified reference materials (CRMs); however, if the bias is very small, the number of sample measurements required to detect it may make the effort impractical.

6.6.4.1 Absolute Bias

Testing for absolute bias is most important when one of the purposes of analysis is to determine whether the analyte is present either in individual laboratory samples or in a sampled population. An absolute bias in the measurement process can lead to incorrect detection decisions. Likely causes of such a bias include inadequate corrections made by the laboratory for instrument background, laboratory reagent contamination, and other interferences.

It is presumed here that the laboratory attempts to eliminate any absolute bias in the measurement process by blank- or background-correcting all measured results. For example, such a correction may be based on measurements of *instrument background* or analyses of *reagent blank* samples. To test whether the corrections are adequate, the laboratory should analyze a series of *method blank* samples, applying all appropriate corrections exactly as for ordinary samples, and perform a *t*-test on the results. To avoid the appearance of a false bias, the determinations of the correction terms (e.g., background or reagent blank) should be repeated for each method blank sample analyzed.

[1] Technically, the laboratory tests the *measurement process* for bias. The measurement process represents the laboratory's implementation of the *method* using particular instruments, analysts, quality control, etc.

6.6.4.2 Relative Bias

Testing the method for relative bias is most important when one of the purposes of analysis is to quantify the amount of analyte present either in a sample or in a sampled population, and perhaps to determine whether the analyte concentration is above or below some positive action level.

To test for relative bias, the laboratory may analyze an appropriate CRM (or spiked sample) a number of times. To avoid the appearance of a false bias, the laboratory should replicate as many steps in the measurement process as possible for each analysis.

6.6.5 Project Method Validation Documentation

Project method validation, depending on the required level of validation, can be accomplished by the project manager sending PT samples to the laboratory or by the laboratory using internal or external PT samples. When PT samples are sent to a laboratory to evaluate or validate the laboratory's method and capabilities, the appropriate technical representative should retain all records dealing with applicable method validation protocols (Section 6.6.2), PT sample preparation certification, level of validation (from Table 6.1), results, and evaluations. Evaluations include comparison of individual results to the validation acceptance criterion, absolute bias in blanks and, if available, statistical analyses of the data for method precision and bias. The laboratory should provide the necessary documentation to the project manager for these PT samples as required by the SOW. The laboratory should request feedback from the project manager as to the method performance. This information, along with the sample analytical results documentation, should be retained by the laboratory for future method validation documentation.

When the laboratory conducts its own project method validation, all records, laboratory workbooks, and matrix spike data used to validate an analytical method should be retained on file and retrievable for a specified length of time after the method has been discontinued. Data evaluations such as comparison of individual results to the validation acceptance criterion and absolute bias in blanks and, when available, method precision and bias, should be part of the data validation package sent to the project manager. All method validation documentation should be retained as part of the documentation related to the laboratory's quality system.

6.7 Analyst Qualifications and Demonstrated Proficiency

The required level of qualification of an analyst is commensurate with the degree of difficulty and sophistication of the method in use. The selection of the analyst for the method application is typically determined initially on experience, education and proven proficiency in similar methods. Basic guidance for the minimum education and experience for radioassay laboratory technicians and analysts has been provided in Appendix E (*Contracting Laboratory Services*) and ANSI N42.23.

For radiochemical methods, there may be several analysts involved. At most major laboratories, different individuals may be involved in the sample preparation, radiochemistry, and radiation detection aspects of the method. In these cases, the entire staff involved in the method should undergo method proficiency tests to demonstrate their ability to meet quality requirements and performance goals. The staff involved in the initial validation of an acceptable method would be considered proficient in their particular role in the method application and the results of their performance should be documented in their training records.

Successful proficiency is established when the performance of the analyst or staff meet predefined quality requirements defined in the laboratory's quality system or a SOW, as well as processing goals. Parameters involved in operational processing goals are typically turnaround time, chemical yields, frequency of re-analyses (percent failure rate), and frequency of errors.

The continued demonstrated analyst proficiency in the method is usually measured through the acceptable performance in internal QC and external PE programs associated with routine sample processing.

6.8 Method Control

Method control is an inherent element of a laboratory's quality system. Simply stated, method control is the ongoing process used to ensure that a validated method continues to meet the expected requirements as the method is routinely used. Method control is synonymous with process control in most quality systems. For a laboratory operation, method control can be achieved by the application of the following:

- Controlled method manual (latest revision and signature sign-off);
- Calibration standards and radiotracers that are traceable to a national standards body such as the National Institute of Science and Technology (NIST) in the United States;
- An instrument QC program that properly evaluates the important method parameters on an appropriate frequency;
- Radiotracers should be evaluated routinely for consistent concentration;
- Chemical yields should be evaluated for trends or deficiencies;
- Internal QC and external PT samples to determine deviations from expected quality performance ranges;
- Standard operating procedures for troubleshooting "out of control" situations; and

- Problem reporting, corrective action, and quality improvement process.

The method control elements described above typically are addressed in the quality manual of the laboratory or the project plan document for the project under consideration. Refer to Chapter 18 for additional information.

6.9 Continued Performance Assessment

The assessment of a laboratory's continued performance is covered in detail in Chapter 7. However, it is important to discuss briefly certain aspects of evaluating a method's continued performance from the perspective of a laboratory.

A performance indicator system should be in place that assesses and provides feedback on the quality of the routine processing. The most useful and cost-effective means of assessing a method's performance is through the implementation of internal QC or external performance evaluation programs or both. Of course, it can be argued that method assessment through a QC or PE program evaluates the combined performance of the method and the analyst. However, statistical and inferential interpretation of the QC/PE data can provide insight into whether the method is failing or whether an analyst is underperforming. Chapters 7 and 18 and Appendix C provides guidance on quality control programs and the use of the internal laboratory QC or external PE data to assess the laboratory's performance in meeting performance criteria.

The laboratory management should use the internal QC program to detect and address radioanalytical issues before the client does. Many SOWs require the use of internal QC samples for every batch of project samples (Chapter 18). In effect, the client is essentially setting the level of internal quality control and the frequency of method performance evaluation. It should be recognized that an internal QC program evaluates method performance related to the initial calibrations or internal "known values." An external NIST-traceable PE program will detect method biases relative to the national standard or to the agency's PE program.

Some users of laboratory services have developed "monitoring" laboratory programs (ANSI N42.23). For these programs, the user engages a recognized independent monitoring laboratory to intersperse double- and single-blind external PT materials into batches of normal samples submitted to a laboratory. The complexity and frequency of the monitoring laboratory PT samples vary among programs, projects, and Federal and state agencies. An external double-blind PE program conducted by a monitoring laboratory using site-specific matrices probably provides the most realistic estimate of the method's or laboratory's true performance. When the monitoring laboratory is traceable to a national standards body (such as NIST in the United States), either directly or through an authorized reference laboratory (ANSI N42.23), the monitoring laboratory program will provide an estimate of any method bias as related to the national standard.

Method performance can also be determined, although on a less frequent basis, through the laboratory's participation in the various PE programs. For a laboratory providing services to government agencies, the participation in such programs is typically a requirement. The PE programs commonly send out non site-specific PT materials on a quarterly or semiannual basis.

The laboratory's performance in certain PE programs is public knowledge. Such information is useful to project managers in selecting a laboratory during the laboratory selection and qualifying processes. Similar to the monitoring laboratory, when the laboratory conducting the PE program is traceable to NIST, either directly or through a NIST reference laboratory (ANSI N42.23), the PE program may provide an estimate of the bias as related to the national standard as well as the precision of the method, depending on the distribution of replicate samples.

Some projects require that all analytical results received from a laboratory undergo a data verification and validation process. Chapter 8 provides more detail on these processes. When properly conducted, certain aspects and parameters of the method can be assessed during the data verification and validation process.

Internal and external audits/assessments are also key elements in a laboratory's quality system to assess the continuing performance of a method (Chapter 7). The level and frequency of the audits and assessments typically vary according to the magnitude and importance of the project and on the performance of the laboratory. Another quality system element that is very effective is a self-assessment program. A functioning and effective self-assessment program may identify weaknesses or performance issues more readily and timely than formal internal and external audits.

6.10 Documentation To Be Sent to the Project Manager

The documentation related to the life cycle of a method application is essentially the information gathered during the use of the method. A formal method documentation program is unnecessary since the information should be part of the quality system documentation. Documented information available from the quality system, related to a method's development, validation, and control, include the following:

- Method validation protocol and results;
- Analyst training and proficiency tests;
- Method manual control program;
- Instrument calibration and QC results;
- Internal QC and external PT sample results;
- Internal and external assessments; and
- Corrective actions.

Data verification and validation information should be kept available and retained for those projects requiring such processes. In addition to QA documentation, the analytical results, either in hard copy or electronic form, should be available from the laboratory for a specified length of time after the completion of a project.

6.11 Summary of Recommendations

- MARLAP recommends the performance-based approach for method selection.
- MARLAP recommends that only methods validated for a project's application be used.
- MARLAP recommends that a SOW containing the MQOs and analytical process requirements be provided to the laboratory.
- MARLAP recommends that the SOW include the specifications for the action level and the required method uncertainty for the analyte concentration at the action level for each combination of analyte and matrix.
- MARLAP recommends that a method undergo some basic general validation prior to project method validation.
- MARLAP recommends that when a method is applied to a specific project, the method should then undergo validation for that specific application.
- MARLAP recommends that as each new project is implemented, the methods used in the analysis of the associated samples undergo some level of validation. However, it is the project manager's responsibility to assess the level of method validation necessary.
- MARLAP recommends a tiered approach for project method validation.

6.12 References

American National Standards Institute (ANSI) N42.23. *Measurement and Associated Instrumentation Quality Assurance for Radioassay Laboratories*. 2003.

American Public Health Association (APHA). 1998. *Standard Methods for the Examination of Water and Waste Water, 20th Edition*. Washington, DC. Available at: www.standardmethods.org.

American Society for Testing and Materials (ASTM) D2777. *Standard Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D-19 on Water*. West Conshohocken, PA.

American Society for Testing and Materials (ASTM) D4855. *Standard Practice for Comparing Methods*. West Conshohocken, PA.

American Society for Testing and Materials (ASTM) E177. *Standard Practice for Use of the Terms Precision and Bias in ASTM Test Methods*. West Conshohocken, PA.

American Society for Testing and Materials (ASTM) E1169. *Standard Guide for Conducting Ruggedness Tests*. West Conshohocken, PA.

American Society for Testing and Materials (ASTM) E1488. *Standard Guide for Statistical Procedures to Use in Developing and Applying ASTM Test Methods*. West Conshohocken, PA.

U.S. Environmental Protection Agency (EPA). 1980. *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Environmental Monitoring and Support Laboratory, Cincinnati, OH. EPA 600-4-80-032, August.

U. S. Environmental Protection Agency (EPA). 2002. *Guidance for Quality Assurance Project Plans.* EPA QA/G-5. EPA/240/R-02/009. Office of Environmental Information, Washington, DC. Available at: www.epa.gov/quality/qa_docs.html.

EURACHEM. 1998. *The Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics*. ISBN 0-948926-12-0. Available at: www.eurachem.ul.pt/index.htm.

International Union of Pure and Applied Chemistry (IUPAC). 2002. "Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis." *Pure Appl. Chem*., 74:5, pp. 835-855.

Youden, W.J. and E.H. Steiner. 1975. *Statistical Manual of the AOAC*. Association of Official Analytical Chemists International, Gaithersburg, MD. Available at: www.aoac.org/pubs/pubcat81.htm.

ATTACHMENT 6A
Bias-Testing Procedure

6A.1 Introduction

This attachment describes a statistical test that may be used to determine whether a laboratory measurement process is biased. The laboratory should check for both "absolute bias" and "relative bias," as defined in Section 6.6.4, "Testing for Bias."

Testing for absolute bias involves repeated analyses of method blank samples. Testing for relative bias requires repeated testing of spiked samples, such as certified reference materials (CRMs) or standard reference materials (SRMs). In either case, it is assumed here that replicate analyses are done at one concentration level, the estimate of which is called the *reference value* and denoted by K. When method blanks are analyzed, the reference value is zero.

6A.2 The Test

Whenever one performs a hypothesis test, one must choose the significance level of the test, which is denoted by α. Most often α is chosen to be 0.05, or 5 percent, but other values are possible. The significance level is the specified maximum acceptable probability of incorrectly rejecting the null hypothesis when it is actually true.

The hypothesis test described below is a *t*-test, modified if necessary to account for the uncertainty of the reference value. The test statistic is denoted by $|T|$ and is calculated by the equation

$$|T| = \frac{|\overline{X} - K|}{\sqrt{s_X^2 / N + u^2(K)}} \tag{6.1}$$

where

- $\overline{X}$ is the average measured value
- s_X is the experimental standard deviation of the measured values
- N is the number of measurements
- K is the reference value (typically K = 0 for method blanks)
- $u(K)$ is the standard uncertainty of the reference value (typically u(K) = 0 for method blanks)

When method blanks are analyzed, $K = u(K) = 0$, and the statistic may be calculated as

$$|T| = \frac{|\overline{X}|}{s_X / \sqrt{N}} \tag{6.2}$$

The number of *effective degrees of freedom* for the T statistic is calculated as follows:

$$\nu_{eff} = (N - 1)\left(1 + \frac{u^2(K)}{s_X^2 / N}\right)^2 \tag{6.3}$$

When $K = u(K) = 0$, the number of effective degrees of freedom is $N - 1$, which is an integer. However, if $u(K) > 0$, then ν_{eff} generally is not an integer[2]; so ν_{eff} should be truncated (rounded down) to an integer. Then, given the chosen significance level, α, the critical value for $|T|$ is defined to be $t_{1-\alpha/2}(\nu_{eff})$, the $(1 - \alpha/2)$-quantile of the t-distribution with ν_{eff} degrees of freedom (e.g., see Table G.2 in Appendix G). So, a bias in the measurement process is indicated if

$$|T| > t_{1-\alpha/2}(\nu_{eff}) \tag{6.4}$$

A measure of the power of this t-test for bias is the *minimum detectable bias* (MDB), which may be defined as the smallest bias (±) that can be detected with a specified probability, $1 - \beta$. The MDB is a function of α, β, N, and the standard deviation of the measured results, σ_X, at the given concentration level. Achieving a small value for the MDB may require the analysis of many replicate samples. If $\alpha = \beta = 0.05$, then at least 16 analyses are needed to ensure the MDB is less than the measurement standard deviation. Fifty-four measurements would be necessary to ensure MDB $\leq \sigma_X / 2$.

EXAMPLE 6.1

Suppose a laboratory analyzes a series of 9 method blanks and obtains the following results (Bq):

0.714 2.453 −1.159 0.845 0.495 0.993 0.472 −0.994 0.673

Determine whether the data indicate an absolute bias. Use a significance level of $\alpha = 0.05$.

Calculate the average of the measured results.

$$\overline{X} = \frac{1}{N}\sum_{i=1}^{N} X_i = \frac{4.492}{9} = 0.49911$$

Note that $\overline{X}$ is the best available estimate of the bias, but it has not yet been determined to be statistically significant.

[2] When the value of ν_{eff} is > 20, one may assume a ν_{eff} value of infinity.

Next calculate the experimental standard deviation.

$$s_X = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(X_i - \overline{X})^2} = \sqrt{\frac{1}{9-1}\sum_{i=1}^{9}(X_i - 0.49911)^2} = \sqrt{1.15455} = 1.0745$$

In this example, the reference value is $K = 0$, with a standard uncertainty of $u(K) = 0$. So, the value of the test statistic, $|T|$, is found as follows.

$$|T| = \frac{|\overline{X}|}{s_X/\sqrt{N}} = \frac{0.49911}{1.0745/\sqrt{9}} = 1.3935$$

Since $u(K) = 0$, the number of effective degrees of freedom is

$$\nu_{\text{eff}} = N - 1 = 8$$

So, the critical value for the statistic is

$$t_{1-\alpha/2}(\nu_{\text{eff}}) = t_{0.975}(8) = 2.306$$

Since $1.3935 \leq 2.306$, no bias is detected.

EXAMPLE 6.2

Suppose a laboratory performs 7 replicate analyses of a standard reference material and obtains the following results (Bq/L):

50.74 53.08 50.73 50.92 51.50 51.11 52.61

Suppose also that the reference value for the SRM is 49.77 Bq/L with a combined standard uncertainty of 0.25 Bq/L.

Determine whether the data indicate a relative bias. Use a significance level of $\alpha = 0.05$.

Calculate the average of the measured results.

$$\overline{X} = \frac{1}{N}\sum_{i=1}^{N} X_i = \frac{360.69}{7} = 51.527$$

Note that the best estimate of the relative bias is $\overline{X}/K - 1$, which equals +0.0353.

Calculate the experimental standard deviation.

$$s_X = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(X_i - \overline{X})^2} = \sqrt{\frac{1}{7-1}\sum_{i=1}^{7}(X_i - 51.527)^2} = 0.94713$$

Calculate the value of the test statistic, $|T|$.

$$|T| = \frac{|\overline{X} - K|}{\sqrt{s_X^2/N + u^2(K)}} = \frac{|51.527 - 49.77|}{\sqrt{0.94713^2/7 + 0.25^2}} = 4.024$$

The number of effective degrees of freedom for the statistic is calculated as follows.

$$\nu_{\text{eff}} = (N-1)\left(1 + N\frac{u^2(K)}{s_X^2}\right)^2 = (7-1)\left(1 + 7\frac{0.25^2}{0.94713^2}\right)^2 = 13.28$$

Note that ν_{eff} is then truncated to 13. Next calculate the critical value for $|T|$.

$$t_{1-\alpha/2}(\nu_{\text{eff}}) = t_{0.975}(13) = 2.160$$

Since $|T| = 4.024 > 2.160 = t_{1-\alpha/2}(\nu_{\text{eff}})$, a bias is detected.

6A.3 Bias Tests at Multiple Concentrations

The discussion above describes a test for bias based on replicate measurements at one concentration level. If replicate measurements are done at each of several concentration levels, the bias test should be performed for each level to evaluate whether there is an "overall" method bias for the entire concentration range based on an a false rejection rate. For this test, the value of α used for each concentration level should be replaced by a smaller value, α′, given by

$$\alpha' = 1 - (1 - \alpha)^{1/m} \tag{6.5}$$

where *m* denotes the number of concentration levels. For example, if the desired overall method false rejection rate is α = 0.05 and the number of levels is three (*m* = 3), the value of α′ for a given test level is 0.01695. When the bias test, using the α′ value, for every concentration level indicates no bias, then the method would be considered free of bias based on an α false rejection rate over the concentration range evaluated. However, this overall method bias test should not be misused or misinterpreted. In some cases, a project manager or laboratory may be more interested to know if a bias exists at one specific test concentration and not at others. For example, the

evaluation of the rate of false- or non-detection for blanks (zero radionuclide concentration) may be more important for a particular project than evaluating the overall method bias for all test levels.

A possible alternative when testing is done at several concentration levels is to use weighted linear regression to fit a straight line to the data and perform hypothesis tests to determine whether the intercept is 0 and the slope is 1. However, determining the most appropriate numerical weights may not be straightforward.