1.4 Method Selection

Refer to Volume 1, Module 2, Sections 5.4.2, 5.4.3, and 5.4.4.

When adding a new analyte to a reference method, the inclusion of the analyte in the method shall meet all required calibration requirements and the QC requirements of the method to which the analyte is being added. If no QC exists in the method, the laboratory shall adhere to the requirements outlined in a reference method of the same technology (when available). -A method that meets these requirements shall be identified in such a way so that there is no confusion that the analyte list has been modified.

1.5 Validation and Verification of Methods

1.5.1 Validation of Methods

Prior to acceptance and institution of any method for which data will be reported, all methods shall be validated for each analyte of interest in each quality system matrix in which data will be reportedto ensure adequacy for its intended use.

1. The laboratory shall validate reference methods via the procedures specified in Sections 1.5.4and 1.5.5.For reference methods, the procedures outlined in Section 1.6 can satisfy the requirements of Section 1.5.5.
2. For all methods, except reference methods, the validation must comply with Volume 1, Module 2, Sections 5.4.5.1, 5.4.5.2, and 5.4.5.3.This validation must include the minimum requirements outlined in Sections 1.5.4, 1.5.4, 1.5.5 and 1.5.6of this module.
3. In cases where the laboratory has been performing an analysis for at least one (1) year prior to applying for accreditation, and there have been no significant changes in instrument type or method, the ongoing method validation data shall be acceptable as initial method validation as long as the ongoing method validation data meets the criteria of 1.5.1 a) or 1.5.1 b) above.
4. For both reference and non-standard methods, laboratories shall perform ongoing method validation for each method, matrix, analyte combination. The results of these ongoing validations shall be used to evaluate the ability of the laboratory to produce acceptable data. On-going method validation may be one of the following:
   * 1. Completion of on-going verification of the DL or LOQ per section 1.5.4
     2. Acceptable performance of a proficiency testing sample
     3. Completion of an Initial DOC per section 1.6.2
     4. Completion of an On-going DOC per section 1.6.3

e) All method validations shall be documented. All data applicable to the method validation shall be retained and readily available at the laboratory.

1.5.2 Initial Verification of Reference Methods

Prior to implementation of any published reference method for which data will be reported, the laboratory’s capability and competency shall be verified for each analyte of interest in each quality system matrix.

1. The laboratory shall validate reference methods using the procedures specified in Sections 1.5.1, and as specified in the reference method, and
2. The laboratory shall have an available analyst that has met the requirement of section 1.6 and associated records and
3. Acceptable performance of proficiency testing samples must be completed, as applicable, see Volume 1 Module 1 for additional information.

1.5.3 Ongoing Method/Matrix/Analyte Verification

1. Laboratories shall perform ongoing verification for each method, matrix, analyte combination annually for all methods, including reference, non-referenceand laboratory developed methods.The results of these ongoing verifications shall be used to evaluate the ability of the laboratory to produce acceptable data. The requirement for On-going verification may be achieved by performing one or more of the following actions, where applicable, for the method/matrix/analyte combination:
2. Completion of on-going verification of the DL or LOQ per section 1.5.4
3. Acceptable performance of a blind sample (single blind to the analyst) or successful analysis of a proficiency testing sample where the analyte has a non-zero assigned value
4. Completion of an Initial DOC per section 1.6.2
5. Completion of an On-going DOC per section 1.6.3
6. When a single action does not provide ongoing verification for each method/matrix/analyte combination (e.g. non-detects in PT samples), actions from above may be combined until a record of verification is available for each method/matrix/analyte combination.

1.5.4 Limit of Detection and Limit of Quantitation (however named)

Procedures used for determining limits of detection and quantitation shall be documented. Documentation shall include the quality system matrix type. All supporting data shall be retained.

1.5.4.1 Detection Limit (DL)

If a mandated test method or applicable regulation includes protocols for determining detection limits, they shall be followed. The laboratory shall document the procedure used for determining the DL. If the method or regulation does not contain specific directions for determination of the detection limit, the following requirements shall apply. DL determinations are not required for methods/analytes for which a detection limit is not applicable such as pH, color, odor, temperature, or dissolved oxygen. DL determinations based on low level spikes are not required for analytesfor which no spiking solutions are available. If results are not reported below the limit of quantitation (LOQ), an initial DL determination is required, but ongoing verification is not.

1.5.4.1.1 Initial determination of the DL

The laboratory DL procedure, unless following a mandated test method or procedure, at a minimum, shall incorporate language addressing the following requirements:

1. the DL shall reflect current operating conditions;
2. the DL determination shall incorporate the entire analytical process including any preparation steps;
3. the DL determination shallinclude data from low level spikes and routine method blanks prepared and analyzed over multiple days; at least one low level spike and routine method blank must be analyzed on each applicable instrument; a minimum of seven (7) replicates is required for both low level spikes and routine method blanks. Both preparation and analysis of these low-level spikes shall include at least three (3) batches on three (3) separate days. Existing data may be used, if spread out over at least three (3) batches in the last twenty-four (24) months.
4. results from low level spikes used in the DL determination shall meet qualitative identification criteria in the method, and shall be above zero;
5. the DL procedureshall include criteria for and evaluation of false positive rates in routine method blanks, DL limits shall not be set below the method blank…;
6. the DL shall be determined for the analytes of interest in each test method in the quality system matrix of interest in which there are neither target analytes nor interferences at a concentration that would impact the results, or the DL shall be performed in the sample matrix of interest.

NOTE: One option is to follow the United States Environmental Protection Agency Method Detection Limit (MDL) procedure, effective September 27, 2017.

1.5.4.1.2 Ongoing verification of the DL

1. At a minimum, ongoing verification of the DL shall include assessments of spikes at or below the LOQ and of method blanks. A minimum of one (1) verification spike and one (1) blank shall be analyzed on each instrument during each quarter in which samples are being analyzed and results are being reported below the LOQ. The criteria listed in Section 1.5.4.1.1 shall be met for ongoing verification over the course of a year.
2. If the method is altered in a way other than routine maintenance and the change can be expected to elevate the detection limit, then a spike at or below the LOQ concentration and a blank shall be prepared and analyzed. If the spike at the LOQ concentration gives a result meeting qualitative identification criteria above zero, and the blank gives a result below the DL, then the DL is verified. If not, the DL shall be re-determined.
3. In the event that verification fails, the laboratory shall perform a new DL study within thirty (30) calendar days.
4. If a new instrument is added to a group of instruments whose data was used in section a) above analyze a minimum of two spikes replicates at the same concentration as the original spikes and at least 2 method blanks. Ensure the method blanks are below the current DL and then recalculate the DL using both the original and new data.

1.5.4.1.3 When a new DL is determined, the laboratory shall verify that the LOQ value is greater than the DL. If it is not, the laboratory shall raise the LOQ value to greater than the DL.

1.5.4.2 Limit of Quantitation (LOQ)

If a mandated test method or applicable regulation includes protocols for determining quantitation limits, they shall be followed. The procedure used for determining the LOQ shall be documented by the laboratory. The laboratory shall select an LOQ for each analyte, consistent with the needs of its clients, and greater than the DL.An LOQ is required for each quality system matrix of interest, technology, method, and analyte, exceptfor any component or property for which spiking solutions are not available or a quantitation limit is not appropriate, such as pH, color, odor, temperature, dissolved oxygen, or turbidity.

1. Each selected LOQ shall be verified through analysis of initial verification samples. An initial verification sample consists of a spiked matrix blank at or below the selected LOQ.
2. All sample processing and analysis steps performed for routine sample analysis shall be included in the LOQ verification testing.
3. The LOQ must be at or above the lowest corresponding calibration standard concentration with the exception of methods using a single point calibration.

d) The laboratory shall establish acceptance criteria for accuracy for the LOQ verification spikes.

1.5.4.2.1 Initial verification of the LOQ  
  
When first establishing an LOQ, or when an LOQ concentration has been selected that is lower than the concentration of the LOQ verification spikes previously performed, an initial verification shall be performed as follows:

a) A minimum ofseven (7) low level spikes at or below the LOQ concentration shall be processedthrough all steps of the method. Both preparation and analysis of these low level spikes shall include at least three (3) batches on three (3) separate days.

NOTE 1: Spiking slightly below the LOQ may help ensure that the results are also suitable for DL determination.

NOTE 2: If low level spikes have been analyzed in order to generate a DL, the results may be used to perform the initial verification of the LOQ.

i. If there are multiple instruments that will be assigned the same LOQ, then these low level spikes shall be distributed across all of the instruments.

ii. A minimum of two (2) low level spikes prepared and analyzed on different days shall be tested on each instrument.

b) Existing data may be used if compliant with the requirements for at least three (3) batches, generated within the last two (2) years and representative of current operations.

c) The LOQ is verified if the following criteria are met:

i. All results are quantitative (above zero and meet the qualitative identification criteria of the method; e.g., recognizable spectra, signal to noise requirements, and presence of qualifier ions).

If a result from an LOQ verification sample is not above zero and/or does not meet the qualitative identification criteria in the method, the problem shall be corrected and the verification repeated, or the LOQ verification shall be repeated at a higher concentration.

ii. The mean recovery of each analyte is within the laboratory established accuracy acceptance criteria.

iii. The LOQ isgreater than the established DL and at or above the spiking concentration.

If the LOQ is less than or equal to the DL, the LOQ shall be raised to greater than the DL.

NOTE: It is **not** necessary to repeat the LOQ verification at a higher concentration when it is necessary to raise the LOQ to greater than the DL.

d) The laboratory shall document the results of the initial LOQ verification as described in Section 1.5.5.4.

1.5.4.2.2 Ongoing verification of the LOQ  
  
The laboratory shall prepare and analyze a minimum of one (1) LOQ verification sample spiked at the same concentration as the initial LOQ verification on each instrument during each quarter in which samples are being analyzed for each quality system matrix, method, and analyte.

1. Results of each LOQ verification sample analysis shall be evaluated at the time of the testing and shall meet the qualitative identification criteria in the method and laboratory Standard Operating Procedure (SOP) and the quantitated result shall be greater than the DL and meet the laboratory established accuracy criteria as established by Section 1.5.2.2 d).
2. If a continuing LOQ verification test does not meet this requirement, the laboratory shall take corrective action and document a technically valid reason for the corrective action. Corrective action shall be one of the following: (i) correcting method or instrument performance and repeating the verification test; (ii) evaluating the laboratory established control limits to ensure they reflect current performance; or (iii) raising the spiking level (and the quantitation limit if the spiking level is above it) and repeating the initial verification study within thirty (30) calendar days of the initial failure. Any samples analyzed in a batch associated with a failing LOQ verification shall be reanalyzed or reported with qualifiers.

1.5.4.3 Verification of DL/LOQ

If no analysis was performed in a given year, the verification of the DL/LOQ is not required, but a new initial DL/LOQ verification shall be performed prior to analysis of client samples.

1.5.4.4 Documentation

At least once per year,the laboratory shall tabulate all results of the ongoing verification sample testing. All data representative of the current operations shall be used, if generated within the last two (2) years. A minimum of seven (7) samples is required.

1. The laboratory shall record the analytical and preparation methods used, dates of preparation and testing, the batch identifiers, the testing instrument, quality system matrix, technology, analyte, concentration in the spiked sample with units, and the test result (if any) for each LOQ and/or DL verification test.
2. For each analyte, the laboratory shall record the percent recovery, the number of results (n), the mean and standard deviation of the percent recovery, and the spiking concentration of the spiked samples with units. These data shall be provided to clients upon request.

1.5.5 Evaluation of Precision and Bias

a) Reference Methods. The laboratory shall evaluate the precision and bias of a reference method for each analyte of concern for each quality system matrix according to Section 1.6 or alternate documented procedure when the analyte cannot be spiked into the sample matrix and QC samples are not commercially available.

b) Non-Reference Methods. For laboratory-developed methods or non-reference methods that were not in use by the laboratory before July 2003, the laboratory shall have a documented procedure to evaluate precision and bias. The laboratory shall also compare results of the precision and bias measurements with criteria established by the client, by criteria given in the reference method or criteria established by the laboratory.

Precision and bias measurements shall evaluate the method across the analytical calibration range of the method. The laboratory shall also evaluate precision and bias in the relevant quality system matrices and shall process the samples through the entire measurement system for each analyte of interest.

Examples of a systematic approach to evaluate precision and bias could be the following:

i. Analyze QC samples in triplicate containing the analytes of concern at or near the LOQ, at the upper-range of the calibration (upper 20%), and at a mid-range concentration. Process these samples on different days as three (3) sets of samples through the entire measurement system for each analyte of interest. Each day, one (1) QC sample at each concentration is analyzed. A separate method blank shall be subjected to the analytical method along with the QC samples on each of the three (3) days. (Note that the three (3) samples at the LOQ concentration can demonstrate sensitivity as well.) For each analyte, calculate the mean recovery for each day, for each level over each day, and for all nine (9) samples. Calculate the relative standard deviation for each of the separate means obtained. Compare the standard deviations for the different days and the standard deviations for the different concentrations. If the different standard deviations are all statistically insignificant (e.g., F-test), then compare the overall mean and standard deviation with the established criteria from above.

ii. A validation protocol, such as the Tier I, Tier II, and Tier III requirements in US EPA Office of Water’s Alternate Test Procedure (ATP) approval process.

1.5.7 Evaluation of Selectivity

The laboratory shall evaluate selectivity by following the checks established within the method, which may include mass spectral tuning, second column confirmation, ICP inter-element interference checks, chromatography retention time windows, sample blanks, spectrochemical absorption or fluorescence profiles, co-precipitation evaluations, and electrode response factors.