New and Alternative Analytical Laboratory Methods

DEP-QA-001/01

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

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TALLAHASSEE, FLORIDA 32399-2400

Bureau of Laboratories

Environmental Assessment Section

(February 1, 2004)

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1.0. Introduction

Department-approved laboratory methods are specified in the Department's program rules, contracts, orders or permits.

Any party may apply to the appropriate Department program for approval for use of a new or alternative laboratory method. The approval of new and alternative methods for use in Department-related work activities is regulated under Rule 62-160.330, F.A.C. Both new and alternative methods shall be demonstrated as appropriate for use as related to the specific data quality objectives of the Department program activity or project for which the method is being used.

> For purposes of this document, "method" refers specifically to an analytical laboratory method. The approval process for new and alternative field sampling methods or procedures is specified in the "Department of Environmental Protection Standard Operating Procedures for Field Activities (DEP-SOP-001/01)".[1]

This document clarifies the specific requirements for demonstrating appropriateness of use. This document also provides guidance on conducting method validation studies and assembling the required method validation documentation package. All method validation packages must be submitted to the following Department section for review:

Florida Department of Environmental Protection
Environmental Assessment Section, MS 6511
2600 Blair Stone Road
Tallahassee, FL 32399-2400
(850) 245-8065

In addition to method validation of the proposed new or alternative method, the method must be certified by the Florida Department of Health's Environmental Laboratory Certification Program prior to use. With the approval of the specific FDEP program for which it will be used, certification for a method developed for a site-specific, limited-use purpose shall be waived.

> **The requirements in this document do not refer to research projects.**
> Research-oriented methodologies are regulated under Rule 62-160.600, F.A.C. However, if a research laboratory method will be used for regulatory purposes, it must undergo validation according to the requirements of this document.

1.1. New and Alternative Methods

Methods that have not been specified in a Department contract, order, permit or Title 62 rule fall into two categories:

- **New Method -** an analytical laboratory method that involves testing for an analyte (chemical compound, component, microorganism, etc.) in a specified matrix where a Department-approved method does not exist.
- **Alternative Method -** an analytical laboratory method that involves testing for an analyte (chemical compound, component, microorganism, etc.) in a specified matrix where a Department-approved method already exists. An alternative method is one intended to be used in place of an existing Department-approved laboratory method.

A **method modification** is any modification to an approved analytical laboratory method that is specifically allowed by the approved method. Method modifications are not considered alternative methods and do not require approval by the Department prior to use. However, the laboratory shall retain all data that demonstrate that the modification produces equivalent results to the unmodified method. These records shall be retained for at least five years after the last use of the modification.

Both new and alternative methods must be validated for use in routine analytical work. In addition, depending on the requirements of the specific program activity for which it is being used, an alternative method must be shown to be equivalent at the 95% confidence level to the one it is intended to replace. The validation requirements for both new and alternative methods depend on their proposed applicability, either as a "limited-use method" or as a "statewide method":

Alternative methods to laboratory methods that have been identified by the United States Environmental Protection Agency as "method defined analyte" (e.g., Method 1311, 1312) will not be accepted.

A laboratory that is using a method that has already been approved for use by a DEP contract, order, permit, Title 62 rule or the United States Environmental Protection Agency does not need to reapply for approval.

1.2. Limited-use and Statewide-Use Methods

Depending on its intended use, a new or alternative method is classified as a "limited-use" or "statewide-use" method:

- **Limited-use Method -** an analytical laboratory method that is validated for the testing of environmental samples from a particular site, waste stream (e.g., facility location), or sample matrix (e.g., effluent, groundwater or drinking water). A limited-use method is validated by a single laboratory and may only be used by that laboratory.
- **Statewide Method -** a laboratory method that is validated for the testing of environmental samples from similar sites or waste streams within the state of Florida by multiple laboratories.

A proposed "limited-use" method only requires data from a single-laboratory study, while a "statewide-use" method requires validation through a multi-laboratory study. For a statewide-use method, the Department requires an interlaboratory collaborative study following guidelines established by the Association of Official Analytical Chemists (AOAC) [2]. Alternatively, when approved by the DEP Environmental Assessment Section, an interlaboratory collaborative study conducted by, or according to, the standards of a recognized consensus-based standards organization (e.g., American Society for Testing and Materials) may be acceptable as a validation means for multi-laboratory methods use.

The flow chart provided in Figure 1.1 clarifies the possible method validation scenarios and directs the reader to the appropriate section in this document containing specific instructions. In addition, three case study scenarios are provided in Appendix B for illustrative purposes.

1.3. Approval of New and Alternative Methods

A new method shall be considered appropriate for use if the Department determines that:

- the information contained in the method validation package supports the quality assurance targets of accuracy, precision, reliability and method detection limit(s) stated by the applicant; and
- the method quality assurance targets meet the stated data quality objectives of the Department contract, order, permit or Title 62 rule for which the method will be used.

An alternative method shall be considered appropriate for use if the Department determines that the technical justification and other submitted information establish that the alternative method provides accuracy, precision, reliability and method detection limit(s) equivalent to, or better than, those of the method it is intended to replace. In addition, an alternative method must be shown to be equivalent at the 95% confidence level to the one it is intended to replace.

> For a listed analyte in an approved method for which the efficiency of the procedure is not published in the method, the laboratory does not have to submit a method validation package to the Department. However, as with any method, the laboratory must conduct appropriate MDL, accuracy and precision studies as specified in sections 2.2.1 and 2.2.2, and a demonstration of capability as required under the NELAC standards. For audit purposes, all documentation related to these studies must be retained on file for five years.

1.4. Methods Proposed for National Pollutant Discharge Elimination System (NPDES) and Safe Drinking Water Act Compliance

If a laboratory proposes an alternative method for analyzing discharges regulated under the federal National Pollutant Discharge Elimination System permit system, the laboratory must comply with provisions of the United States Environmental Protection Agency through 40 CFR Part 136 paragraphs 136.4 and 136.5 (2000). Applicants must submit the application to the Department, which shall forward the application to the United States Environmental Protection Agency Administrator of Region 4 for review and approval. The determination for approval or rejection shall be made by the United States Environmental Protection Agency.

If a laboratory proposes an alternative method for analyzing compliance samples under the federal Safe Drinking Water Act, the laboratory must comply with provisions of the United States Environmental Protection Agency (40 CFR Part 141 paragraph 127) and Department Rule 62-550.550, F.A.C. Use of an alternative analytical technique requires written permission from the Department's Drinking Water Section and the United States Environmental Protection Agency. Applicants should contact the FDEP Drinking Water Section for details.

Figure 1.1: Decision-making - New and Alternative Methods for Statewide or Limited-Use

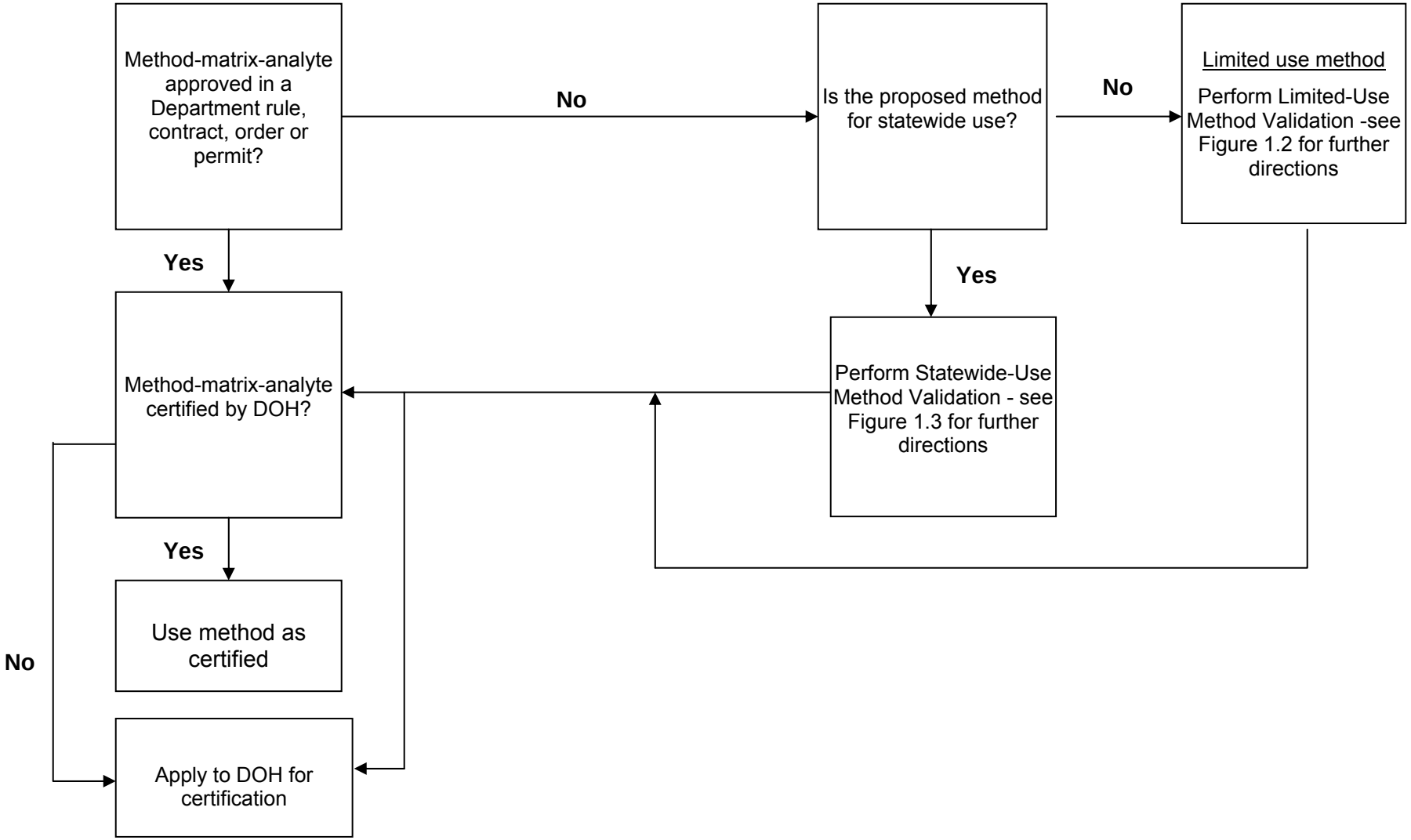


Figure 1.2: Decision-making – Limited-Use Method Validation Flow Diagram

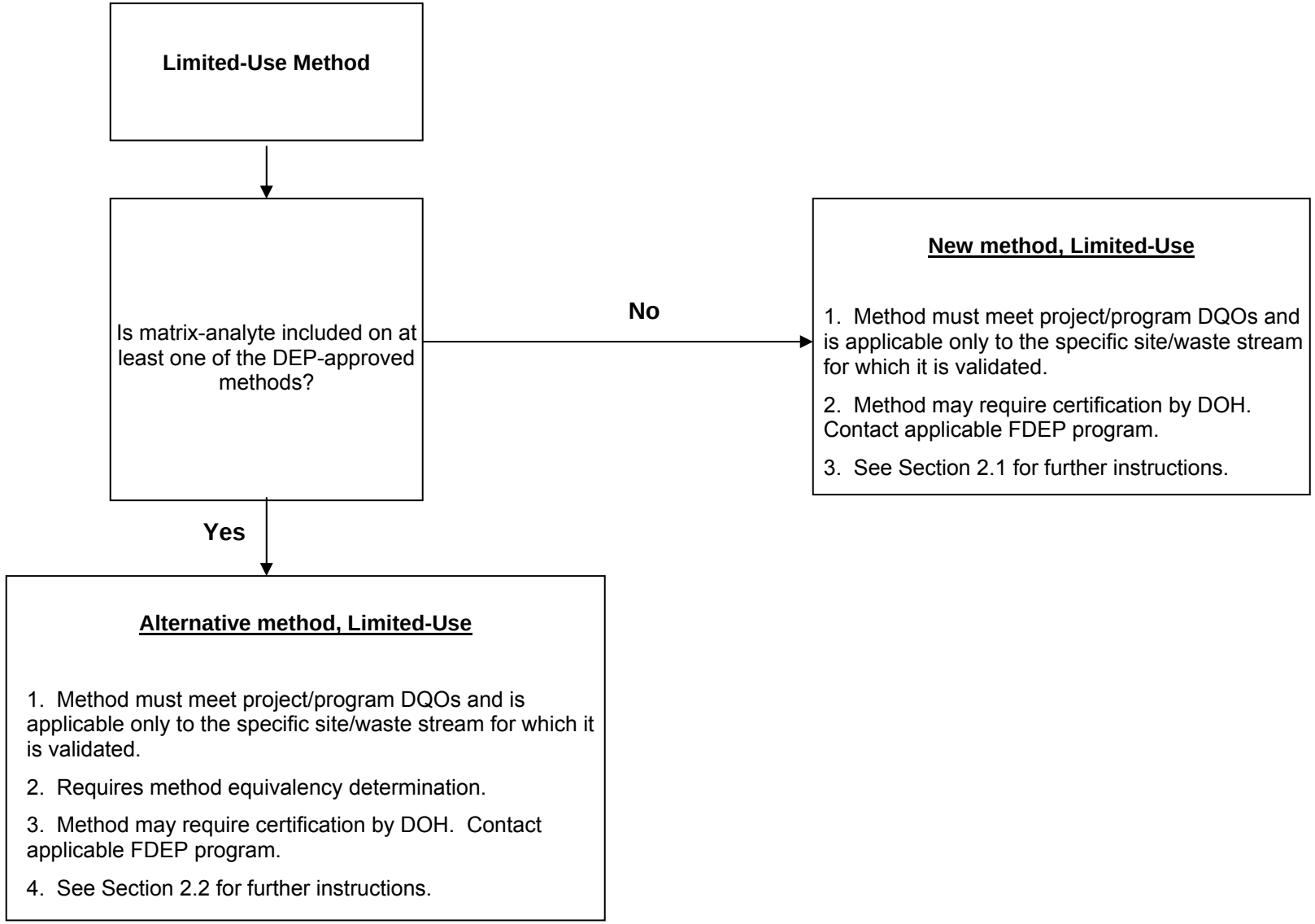
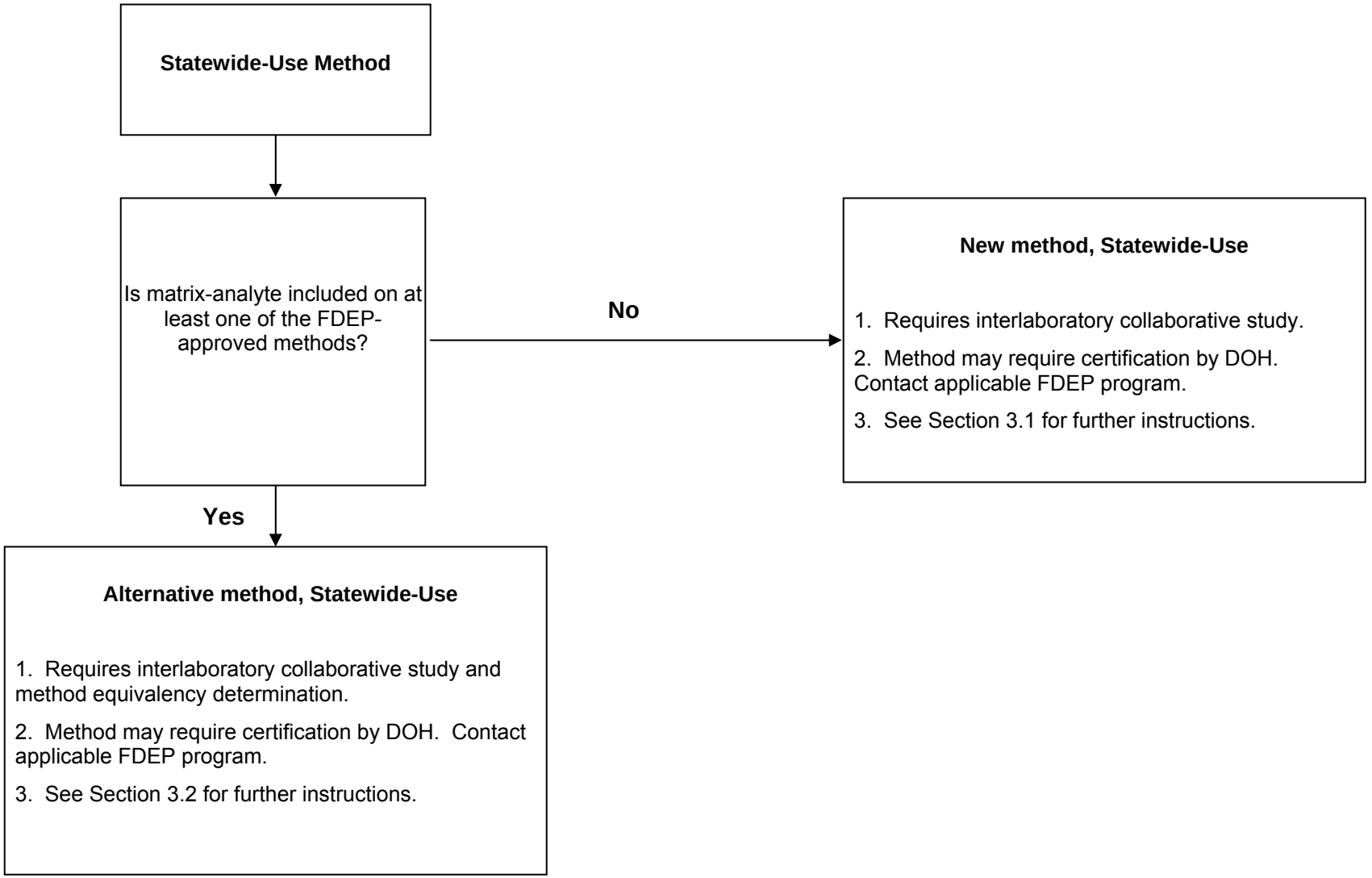


Figure 1.3: Decision-making – Statewide-Use Method Validation Flow Diagram



2.0. Requirements for New and Alternative Analytical Laboratory Methods for Limited Use

2.1. New Method for Limited Use

A new method tests for an analyte (chemical compound, component, etc.) in a specified matrix where a Department-approved method does not exist. If the method is to be used only by a single laboratory, it is designated as "limited use". For a limited-use method, the laboratory must conduct the following studies (refer to **Appendix B-1** for a detailed case scenario):

2.1.1. Method Detection Limit (MDL) Determination

- If the method or regulatory program does not specify a protocol for determining the MDL, use one of the protocols specified in **Appendix C-2** to calculate the MDL.
- Use analyte-free water (high quality reagent water, Standard Methods, 20th Ed. part 1080)[3] as the sample matrix.
- Use all target analytes and any proposed surrogates (if applicable) for the fortification (spike) compounds.
- Calculate the MDL using the statistics and formulae of the selected MDL method.

NOTE: The above study (a) is not necessary if it was conducted during the method development stage and if the results are supported by the published journal article.

2.1.2. MDL, Precision and Accuracy Determinations in the applicable sample matrix

- Use the appropriate matrix applicable to the method being validated (e.g., drinking water, soil, groundwater, etc.). The matrix selected must be free of target compounds (i.e., analytes below the MDL of method);
- Fortify spiked samples with all target analytes and proposed surrogates and/or internal standards (if applicable);
- Conduct the study using the same method that was used to determine the MDL in 2.1.1. above.
- Refer to Appendix C-1 for appropriate formulae for calculating Accuracy (as % Recovery) and Precision (as % RSD); use the statistics and formulae of the selected MDL method to determine the MDL.

2.1.3. Required Documentation: the Method Validation Package (MVP)

The laboratory must submit the following documentation (items a-g) to the FDEP Environmental Assessment Section for review and approval:

a. Name(s), mailing address and telephone number of individual(s) preparing the package: a cover letter will satisfy this requirement. The letter must contain a statement requesting approval of the referenced method for limited use application.

b. A statement about the reason for, and the applicability of, the new, limited-use method. This statement must identify the specific Department project or program activity for which the method will be used.

c. A complete description of the proposed new method. A copy of the research paper, if applicable, may be included but it cannot replace the required method description (i.e., step-by-step procedure). The description must include the following:

- **Title Page**

- Identify the Method (e.g., *Analysis of XYZ Pesticide and its Metabolites in Groundwater by HPLC*).

- **Scope and Application**

- Describe the scope and applicability of the method including the matrix or matrices.

- Include a list of the applicable analyte(s), the limits of detection or concentration ranges for each, and any precautionary notes.

- **Summary of Method -** Give a brief description of the method, including sample preparation, type of instrumentation used, detectors, confirmation requirements; and types of standards used (internal/external), etc.

- **Definitions -** Define any terms that may not be commonly understood, or that have multiple meanings.

- **Interferences**

- Discuss interferences that may result from processing and analysis of samples (e.g., from solvents, reagents, glassware and other sample processing hardware).

- Discuss procedures necessary to reduce or eliminate such interferences (e.g. glassware cleaning).

- Discuss matrix interferences and how to reduce or compensate for their effects.

- **Safety -** Address all safety aspects of sample handling and processing (e.g., OSHA regulations, health effects of chemicals used and precautionary measures).

- **Apparatus and Materials -** Describe or identify all analytical equipment and materials. This requirement includes sample containers, glassware and ancillary equipment (i.e., water baths, balances, etc.).

- **Reagents and Standards -** Describe the preparation of all reagents and standards. Include precautions and/or specifications for reagent and standard grades.

- **Calibration -** Describe the procedures for initial calibration of the method, the method for generating the calibration curve (for example, linear regression, quadratic fit, etc.) and the acceptance criteria.

- **Quality Control -** Address all QC measures needed for initial demonstration of capability and for routine analysis. Include frequency and all acceptance criteria.
- **Sample Collection, Preservation and Handling -** Address sample type (grab, composite), required container and preservation, maximum holding times and any special precautions that might be needed when collecting the samples.
- **Sample Extraction/Preparation -** Describe the protocols used to extract, digest or prepare the sample prior to analysis.
- **Sample Cleanup and Separation -** Describe any protocols needed to separate the analyte(s) of interest from the matrix.
- **Sample Analysis -** Describe all protocols relating to sample analysis. Include instrument conditions, column type (if applicable), solvent or temperature programs, etc.
- **Calculations -** Include all formulas used in calculating final concentrations.
- **Confirmation -** Include protocols used to confirm the presence of the analyte (for example, GC/MS, second column, alternative wave length, etc.).
- **Data Assessment -** Include all procedures to be used in assessing the data including quality control acceptance criteria.
- **Corrective Actions -** Include all measures that will be taken if a quality control measure or other measures of performance are not acceptable. Discuss contingencies for handling unacceptable data.
- **Method Performance -** In a table format, summarize the method detection limit, quality control acceptance ranges and other pertinent information (for example, retention times, extraction/cleanup efficiency, etc.).
- **Pollution Prevention and Waste Management -** Include all measures to prevent pollutions, and how waste products (extracts, digestates, etc.) are to be handled.
- **Tables, Diagrams, Flowcharts -** Include any applicable tables or figures.
- **Validation Data -** Include or provide specific location of all validation and initial demonstration of capability data.
- **References -** include any applicable reference citations.

d. Copies of raw data: sample and standards preparation logs, chromatograms (blanks, standards, spiked samples), analysis/instrument logs, etc.

e. Copies of QC data: QC check standards, continuing calibration verification standards (CCVS), surrogates, etc.

f. All calibration data: concentration of standards, calculation of response factors and calibration curves (if linear regression is applicable, include the acceptance criteria for the resulting regression coefficient).

g. All calculations pertaining to items 2.1.1 and 2.1.2 (i.e., MDL, precision and accuracy determinations).

2.2. Alternative Method for Limited Use

An alternative method for limited use is one used by a single laboratory in place of an existing Department-approved method. The laboratory must conduct the following studies (refer to **Appendix B-2** for a detailed case scenario):

2.2.1. Method Detection Limit (MDL) Determination

- If the method or regulatory program does not specify a protocol for determining the MDL, use one of the protocols specified in **Appendix C-2** to calculate the MDL.
- Use analyte-free water (high quality reagent water, Standard Methods, 20th Ed. part 1080)[3] as the sample matrix.
- Use all target analytes and any proposed surrogates (if applicable) for the fortification (spike) compounds.
- Calculate the MDL using the statistics and formulae of the selected MDL method.

NOTE: The above study (2.2.1) is not necessary if it was conducted during the method development stage and if the results are supported by the published journal article.

2.2.2. MDL, Precision and Accuracy Determinations in the Applicable Sample Matrix

- Use the appropriate matrix applicable to the method being validated (e.g., drinking water, soil, groundwater, etc.). The selected matrix should either be free of target compounds (i.e., analytes below the MDL of method) or have very low concentrations of the target compounds.
- Fortify spiked samples with all target analytes and proposed surrogates and/or internal standards (if applicable).
- Conduct the study using the same method that was used to determine the MDL in 2.2.1 above.
- Refer to Appendix C-1 for appropriate formulae for calculating Accuracy (as % Recovery) and Precision (as % RSD); use the statistics and formulae of the selected MDL method to determine the MDL.

2.2.3. Equivalency Study (if required)

If required for a specific Department program activity, an alternative method must be shown to be equivalent at the 95% confidence level to the one it is intended to replace. The following study must be conducted to demonstrate this equivalency:

Using the relevant sample matrix (groundwater, surface water, soil, etc.) shown to be free of the analytes of concern, the laboratory should prepare a minimum of seven (7) replicates for both methods. The spiking level must be at the estimated PQL of the approved method.

Note: The number of replicates is dictated by the determination of MDLs (for example, n=7 replicates, if the Appendix B of 40 CFR 136 is followed).

The same analyst/workgroup may conduct the analyses or two persons/workgroups may participate: one for the alternative method and one for the approved method. Preferably, all laboratory work (including sample extractions) should be carried out in the least amount of time (e.g., within 48 hours of sample spiking). Equivalency is shown if the MDL, PQL, precision and accuracy of the alternative method are comparable (i.e. statistically equal at the 95% confidence level, CL) to, or better than, the same quality assurance indicators in the "standard" method.

STATISTICAL EVALUATION

The 95% confidence interval estimate of the MDL is derived from percentiles of the chi square over degrees of freedom distribution (see Appendix B of 40 CFR 136). In the case of 7 replicates, the lower and upper control limits (LCL and UCL) of this skewed interval are calculated as:

Practical Quantitation Limit **LCL = 0.64 x PQL** **UCL = 2.20 x PQL**

Method Detection Limit **LCL = 0.64 x MDL** **UCL = 2.20 x MDL**

The 95% confidence interval of the accuracy (as %R) is directly proportional to the 95% confidence interval of the mean value X, which is calculated using the Students' T distribution factors. In this case, the interval for <%R> is symmetric and can be calculated as:

Accuracy **LCL = <%R>[1 - 0.0093 x (%RSD)]**

UCL = <%R>[1 + 0.0093 x (%RSD)]

Equivalency of the two methods is verified if

1. the corresponding intervals for MDL and PQL are either lower than or overlap with the corresponding intervals of the approved method and

2. the accuracy interval <%R> of the alternative method overlaps the corresponding interval of the approved method.

2.2.4. Required Documentation: the Method Validation Package (MVP)

The laboratory must submit the following documentation (items a-f) to the FDEP Environmental Assessment Section for review and approval:

a. Name(s), mailing address and telephone number of individual(s) preparing the package: a cover letter will satisfy this requirement. The letter must contain a statement requesting approval of the referenced method for limited-use application.

b. A statement specifying the reason for using a method other than one specified in a Department rule, contract, order or permit. This statement must clearly specify the justification for using the alternative method and must state the FDEP-approved method that the alternative method will replace. This statement must also identify the specific Department project activity and for which the method will be used.

c. A complete description of the new method: see 2.1.3 item c.

d. A description of the method study(ies).

e. Reduced and raw analytical data for each method: see 2.1.3 items d-g.

f. If an equivalency study is required, include the raw data, calculations and the complete description of statistical analysis as specified in section 2.2.3.

3.0. Requirements for New and Alternative Analytical Laboratory Methods for Statewide Use

3.1. New Method for Statewide Use

A new method for statewide use is one that:

- Tests for an analyte (chemical compound, component, etc.) in a specified matrix where a Department-approved method does not exist.
- Is validated for testing environmental samples from similar sites or waste streams within the state of Florida by multiple laboratories.

For a statewide-use method, the Department requires an interlaboratory collaborative study following the specifications in Appendix D of the "Official Methods of Analysis of the Association of Official Analytical Chemists"[2]. Alternatively, an interlaboratory collaborative study conducted by a recognized consensus-based standards organization (e.g., American Society for Testing and Materials) may be used as a validation means for multi-laboratory methods use. **Prior to conducting the interlaboratory collaborative study, contact the FDEP Environmental Assessment Section to ensure the methodology used is acceptable.**

The Department recommends a method be subject to the limited-use validation described in section 2.1 before it undergoes a validation study for statewide application. This is not necessary if the MDL(s) for the analyte(s) in the relevant matrix have been determined using one of the three protocols noted in Appendix C.

3.1.1. Any organization requesting the approval for use of new method for statewide use (i.e., use by multiple laboratories throughout the state) must follow these requirements:

a. One of the participating laboratories must serve as, or be affiliated with, the referee organization. The referee organization:

- Distributes the analytical procedure to be followed by all laboratories.
- Prepares the replicate samples (e.g. spiked groundwater aliquots) and supplies them to the other laboratories,
- Receives all analytical results and performs the appropriate statistical calculations.
- Assembles the final application package submitted to the FDEP Environmental Assessment Section.

b. A minimum of 8 (eight) laboratories must participate in, and report valid data for, the interlaboratory collaborative study. In special cases involving expensive equipment or highly specialized laboratory services, the study may be conducted with a minimum of five (5) laboratories, with the approval of the FDEP Environmental Assessment Section.

c. A minimum of 5 (five) samples must be analyzed by each of the participating laboratories.

d. Appropriate statistical tests must be used in the analysis of the reported analytical data. Normal or parametric statistics are generally sufficient to test for

the significance of the data and for carrying out the analysis of variances. The following measures of variability must be evaluated:

- Within-lab variance;
- Between-lab variance; and
- Overall variance of the data.

The reproducibility of the data at each concentration level (generally two, as recommended by AOAC) must be less than the pre-established critical value (e.g., regulatory limit) for the method to be validated. The AOAC guidelines ensure a low chance of acceptance for poor methods and a moderate chance of rejection for good methods.

3.1.2. Experimental Studies

The following experimental protocol must be followed:

a. Use the sample matrix equivalent to that for which the method is being proposed (e.g., groundwater, soil, etc.). Sample matrix must be free of target compounds;

b. Spike compounds: all target analytes and proposed surrogates and/or internal standards (if applicable);

c. Spike concentrations: fortify half of the samples at 1-2 times the calculated PQL **(Low Level).** Fortify the remaining samples at 10 times the chosen low level spike **(High Level)** or at a concentration at the high end of the calibration range;

d. Provide to each participating laboratory at least one set of sample replicates fortified at the **Low Level**, and one set fortified at the **High Level**. All samples must be submitted to the laboratories as blind samples;

e. Each laboratory must use the proposed methods to measure one low and one high level sample per day (thus all analyses are done over a four-day period); and

f. All participating laboratories must report their raw data and calculated concentrations for each sample to the designated referee organization.

3.1.3. Statistical Evaluation of Sample Data

The referee organization must conduct the statistical evaluation specified in section 6.3, Appendix D of the "Official Methods of Analysis of the Association of Official Analytical Chemists" [2] unless an equivalent statistical evaluation from a consensus organization is proposed and approved for use.

If the method is found acceptable, the application package to FDEP may request that it be made available to other laboratories, upon request, as an approved method. If the method is rejected (i.e. does not meet minimum acceptance criteria), the application package to FDEP may include suggestions for method improvement or that a limited-use status be considered for the proposed method.

3.1.4. Required Documentation: the Method Validation Package (MVP)

Submit the following documentation to the FDEP Environmental Assessment Section:

- Name(s), mailing address and telephone number of individual(s) preparing the package: a cover letter will satisfy this requirement. The letter must contain a statement requesting approval of the referenced method for statewide application.
- A complete description of the method: see 2.1.3, item c.
- A description of the method validation study identifying all participating laboratories.
- Reduced and raw analytical data for each laboratory: see 2.1.3, items d-g.
- A complete description of statistical analysis, according to section 3.1.3, and associated conclusions.

3.2. Alternative Method/Statewide-Use

An alternative method for statewide use is one that:

- Tests for an analyte (chemical compound, component, etc.) in a specified matrix where a Department-approved method does exist. An alternative method is one intended to be used in place of an existing Department-approved method.
- Is validated for testing environmental samples from similar sites or waste streams within the state of Florida by multiple laboratories.

Using the previous examples in Sections 2.2 and 3.1, the laboratory must design an appropriate validation study for its particular case. The Department encourages laboratories to consult with the staff of the Environmental Assessment Section to discuss study designs in this category.

Appendix A: Glossary

Alternative Method - one that tests for an analyte (chemical compound, component, microorganism, etc.) in a specified matrix where a Department-approved method does exist. An alternative method is one intended to be used in place of an existing Department-approved method.

Audit - a systematic review of laboratory and field procedures to determine if proper procedures are being used and supporting documentation is present. An audit must consist of an on-site assessment of sample collection, field sampling procedures, laboratory procedures and/or a review, assessment and/or validation of data associated with a Department program activity. If necessary, an audit shall include the submission of performance samples (for example, blind, split and/or performance check samples) to an organization for subsequent use in the evaluation of that organization's technical performance associated with a specific Department project or program activity.

Data Quality Objectives - a set of qualitative and quantitative requirements that environmental data must achieve to be acceptable for use in a specific program or project. The requirements pertain to the quality of the data in terms of precision, accuracy, sensitivity, selectivity, representativeness and comparability.

Data Validation - means an evaluation of the technical usability of the verified data with respect to the planned objectives or intention of a project.

Data Verification - is a consistent, systematic process that determines whether the data have been collected in accordance with project specifications with respect to compliance, correctness, consistency and completeness as compared to a method standard or contract specification.

Department - the Florida Department of Environmental Protection, also referred to as "FDEP".

Florida Department of Health's Environmental Laboratory Certification Program (DOH ELCP) - a laboratory certification program recognized by the National Environmental Laboratory Accreditation Program (NELAP) as an authority with responsibility and accountability for granting accreditation for specified fields of laboratory testing. The standards used by the DOH ELCP are those established by the National Environmental Laboratory Accreditation Conference (NELAC) as specified in Chapter 64E-1, F.A.C.

Limited-Use Method - a laboratory procedure that is validated for testing environmental samples from a particular site, waste stream (e.g., facility location), or sample matrix (e.g., effluent, groundwater, or drinking water). A limited-use method is validated by a single laboratory and may only be used by that laboratory.

Method Detection Limit (MDL) - the minimum concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. For an MDL study, all sample processing steps of the analytical method shall be included. MDLs must be determined following procedures specified in the "New and Alternative Analytical Laboratory Methods, DEP-QA-001/01" unless otherwise specified by a mandated test method for which the laboratory is certified or seeking certification.

Method Modification - any modification to an approved field procedure or analytical laboratory method that is specifically allowed by the approved field procedure or analytical laboratory method. Method modifications are not considered alternative methods and do not require approval by the Department prior to use. However, the laboratory or field sampling organization, as applicable, must retain on file for five years any required data specified by the

method that demonstrate the method modification produces equivalent results when applied to the relevant sample matrix.

Method Validation - the process by which a laboratory establishes the performance of a new method or substantiates the performance of an alternative method. New and alternative methods must be validated to prove that they accurately measure the concentration of an analyte in an environmental sample. A method validation package is the documentation package (e.g., raw data, calculation, method description) supporting the method validation.

National Environmental Laboratory Accreditation Conference (NELAC) - a voluntary organization of state and federal environmental agencies formed to establish and promote mutually acceptable performance standards for the operation of environmental laboratories. These standards cover both analytical testing of environmental samples and the laboratory accreditation process. The goal of NELAC is to foster the generation of environmental laboratory data of known and documented quality through the development of national performance standards for environmental laboratories and other entities directly involved in the environmental field measurement and sampling process.

National Environmental Laboratory Accreditation Program (NELAP) - is the program that implements the NELAC standards.

NELAP Accreditation - is an accreditation status applied to a laboratory's field(s) of testing upon satisfying all requirements for certification as provided in Chapter 64E-1, F.A.C.

New Method - one that tests for an analyte (chemical compound, component, microorganism, etc.) in a specified matrix where a Department-approved method does not exist.

Practical Quantitation Limit (PQL) - the lowest level that can be reliably achieved during routine laboratory operating conditions within specified limits of precision and accuracy. If a laboratory fails to report a PQL, the PQL will be calculated as four times the MDL.

Quality Assurance - an integrated system of activities involving planning, quality control, quality assessment, reporting and quality improvement to ensure that a product or service meets defined standards of quality with a stated level of confidence.

Quality Control - is defined as the overall system of activities whose purpose is to control the quality of environmental data so that they meet the data quality objectives established by the users.

Rejection of data - the Department shall not use the data for the program or project for which they were generated. If the data do not comply with the validation criteria specified in Rule 62-160.670(1), F.A.C., they shall be subject to rejection in part or in whole for use by Department programs, as provided in Rule 62-160.670(2), F.A.C.

Research Method - a field or laboratory procedure that involves the evaluation or use of new, innovative technology.

Statewide-Use Method - a laboratory procedure that is validated for testing environmental samples from similar sites or waste streams within the state of Florida by multiple laboratories.

Appendix B: Case Studies

B-1. New Method for Limited Use

- XYZ Laboratories proposes a new method for use by its own laboratory (limited use) to analyze for a new pesticide and its two metabolites in groundwater.
- There is no approved procedure for the analysis of the pesticide.
- The laboratory proposes to use an HPLC method developed in-house. A research paper has been published in a refereed journal describing the method development. In addition, the results from an MDL study in reagent water are published in this referred journal.

a. XYZ Laboratories conducts the following studies:

1. MDL Determination in Reagent Water

Because the results from an MDL study were published in a refereed journal, the laboratory chooses to bypass the MDL determination in reagent water.

2. MDL Determination in Groundwater

- XYZ Laboratories elects to use the EPA protocol (40 CFR part 136 Appendix B) as their MDL calculation method.
- The laboratory uses groundwater they have analyzed and determined to be free of their target compounds (i.e. all target compounds are below published MDLs).
- The laboratory prepares seven replicate spiked groundwater samples. Using the published MDL values and the laboratory's own determined instrument detection limits for the pesticide and its metabolites, the seven samples are fortified at the following levels:

	MDL (ug/L)
Pesticide A	5
Metabolite B	10
Metabolite C	20

- In addition, the lab fortifies the sample with two surrogates it is proposing to use in this new method.
- XYZ Laboratories calculates the standard deviation of the seven replicate samples for the target pesticide and its two metabolites using the EPA protocol (40 CFR Part 136 Appendix B). The laboratory uses these standard deviation values to calculate the final MDL (that is, the MDLs are three times the determined standard deviation). The results are:

	Standard Deviation (S), as calculated from 40CFR Part 136 Appendix B	MDL (3 x S) (ug/L)
Pesticide A	0.82	2.5
Metabolite B	2.4	7.2
Metabolite C	5.6	16.8

3. XYZ Laboratories assembles the validation package containing all elements cited in **Section 2.1.3** above.

B-2. Alternative Method for Limited Use

EnviroX Laboratories proposes an HPLC method for the determination of pesticide Y in groundwater as an alternative to an existing FDEP-approved GC method for the determination of the same pesticide in groundwater. The laboratory conducts the following studies:

a. Using a groundwater sample matrix free of the target compound (Pesticide Y), the laboratory prepares 14 (fourteen) spikes at 3-5 times the MDL of the reference GC method. Spike compounds include Pesticide Y and the proposed surrogates and the internal standards common to both methods.

b. EnviroX analyzes half of the spikes by each method and calculates the matrix-specific MDL, precision and accuracy, using formulas in Appendix A above, for each set of seven replicates. The resulting values are:

	MDL (ug/L)	Accuracy (% R)	Precision (%RSD)
DEP-Approved (Standard) GC Method	10	75	10
Proposed Alternative HPLC Method	17	87	8

c. EnviroX carries out the statistical evaluation described in Section 2.2.3. The table below shows a comparison of the confidence intervals:

	Alt. HPLC Method		**Standard GC Method**		
Equivalent Quantity	LCL	UCL	LCL	UCL	(Yes/No)
MDL	11 µg/L	37 µg/L	6.4 µg/L	22 µg/L	Yes
<%R>	68%	82%	80.5%	93.5%	Yes

Therefore, in this example, the two methods are shown to be equivalent and the HPLC method would be approved as an alternative to the GC method.

d. EnviroX assembles the validation package containing all elements cited in Section 2.2.4 above and submits the complete package to the Department for review and approval prior to use.

B-3. New Method for Statewide Use

- XYZ Laboratories decides to propose its newly developed HPLC method for statewide use. This means the proposed method, when validated, will be available to any laboratory that wishes to use it in the analysis of groundwater. The DEP has recently approved the method for limited-use (see B-1 above for details)
- Eight laboratories will participate in the validation of the new HPLC method: XYZ Laboratories (the Referee Lab), plus laboratories A, B, C, D, E, F and G.
- The MDL(s) for groundwater were calculated during the method development stage (see B-1 above).

a. XYZ Laboratories proceeds as follows:

1. Each laboratory must receive five negative controls and ten replicate samples, five at a low level concentration and five at a high level concentration.

2. Using groundwater free of the target compounds, XYZ fortifies all samples with target compounds and the two proposed surrogates. Half of the samples are spiked at 3-5 times the calculated MDLs (Low Level) and the other half are spiked at a level 10 times the chosen low level spike (High Level).

3. Each laboratory uses the proposed methods and measures one low and one high level sample per day (thus all analyses are done over a four-day period).

4. The laboratories report their raw data and calculated concentrations for each sample to the XYZ (the Referee Laboratory).

5. XYZ Laboratories performs the statistical evaluation according to above Section 2.2.4. XYZ assembles the complete method validation package according to above Section 2.2.4 and submits this package to the FDEP for review.

Appendix C: Calculations and Applicable Formulae

C-1. Formulas for Calculating Precision and Accuracy

Use the following formulas for calculating the precision and accuracy of test measurements and the associated acceptance ranges:

a. **PRECISION**

Calculate the precision of replicate samples using one of the following three formulas:

1. PERCENT RELATIVE STANDARD DEVIATION: **(% RSD) = (S÷X)x100 (%)**

   Where: X = Mean (average) of the data points
   s = Standard deviation calculated as:

$$s = \{[\Sigma(X-Xi)^2]\div(n-1)\}^{0.5}$$

   X = mean value of measured concentrations (µg/L)
   Xi = value of each measured concentration (µg/L)
   n = number of determinations

2. RELATIVE PERCENT DIFFERENCE: **(RPD) = $\frac{A-B}{A+B}$ x 200 %**

   Where: A = concentration in sample A
   B = concentration in sample B

3. INDUSTRIAL STATISTIC: $(I) = \frac{A-B}{A+B}$

   Where: A = concentration in sample A
   B = concentration in sample B

b. **Accuracy (as % Recovery)**

Determine the accuracy (as % recovery) by calculating the % recovery of a known amount of analyte from a the fortified sample as follows:

$$\%\ R = \frac{\text{Spiked Sample Concentration} - \text{Unspiked Sample Concentration}}{\text{Fortification Concentration}} \times 100\ (\%)$$

C-2. Method Detection Limits and Practical Quantitation Limits

a. **Method Detection Limits (MDLs)**

The Method Detection Limit (MDL) is the minimum concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte. For an MDL study, all sample processing steps of the analytical method must be included (sample preparation, sample clean-up, sample analysis, etc.). The MDL must be determined following one of the procedures specified below, unless otherwise specified by a mandated test method for which the laboratory is certified or seeking certification through the Florida Department of Health's Environmental Laboratory Certification Program.

The MDL must be calculated according to the requirements of the selected method.

The MDL is not the same as the Instrument Detection Limit (IDL) which must never be used in place of the MDL. The IDL is determined using multiple analyses of standards and is useful in determining an experimental concentration level to use when fortifying samples for MDL determination. The MDL is determined by processing samples through the ENTIRE analytical procedure (not just analysis).

The laboratory must determine the MDL using the protocol specified in the approved test method or applicable regulation. If the protocol for determining detection limits is not specified, use one of the following three protocols:

- EPA - "Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11", 40 CFR Part 136, Appendix B [4];
- IUPAC- "Nomenclature in Evaluation of Analytical Methods including Detection and Quantification Capabilities", Pure & Appl. Chem., Vol. 67, No. 10, pp. 1699-1723, 1995 [5].
- Hubaux and Vos- "Decision and Detection Limits for Linear Calibration Curves", Analytical Chemistry, Vol. 42, No. 8, July 1970, pp. 849-855 [6]

Both the more prescriptive method published in Chapter 40, Code of Federal Regulations (40 CFR) and the method endorsed by the International Union of Pure and Applied Chemistry (IUPAC) are derived after the method published by Lloyd Currie[9] and assume a constant error model within a small concentration region. These methods set the MDL at a critical value intended to exclude 99% of the analytical noise population from reportable levels, yet one drawback to the 40 CFR method is that analytical artifacts leading to bias in the noise distribution are not considered. The method published by Hubaux and Vos, on the other hand, is based on a variable error model and the effect of concentration on the resulting noise distribution is considered in determining the detection limit. While this technique is more robust than that of other models, considerably more effort is required to develop method detection limits.

Any methods that support compliance monitoring and reporting for EPA's National Pollutant Discharge Elimination System (NPDES) program must use the 40 CFR method.

b. **Practical Quantitation Limit (PQLs)**

The Practical Quantitation Limit (PQL) is the lowest level that can be reliably achieved during routine laboratory operating conditions within specified limits of precision and accuracy. Typically, the PQL is 3-5 times the MDL, and it "represents a practical and routinely achievable detection level with a relatively good certainty than any reported value is reliable" [4].

If a laboratory fails to report a PQL, the PQL will be calculated as 4 times the stated MDL.

Appendix D: References

1. Department of Environmental Protection Standard Operating Procedures for Field Activities (DEP-SOP-001/01). Florida Department of Environmental Protection. (February 1, 2004).
2. "Interlaboratory Collaborative Study for Method Validation in the AOAC", Appendix D, Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC), 16th edition, Association of Official Analytical Chemists, (1995).
3. Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association, American Water Works Association and Water Environment Federation Standard Methods for the Examination of Water and Wastewater, (1998).
4. EPA - "Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11", 40 CFR Part 136, Appendix B;
5. IUPAC- "Nomenclature in Evaluation of Analytical Methods including Detection and Quantification Capabilities", Pure & Appl. Chem., Vol. 67, No. 10, pp. 1699-1723, 1995 [6].
6. Hubaux, A., G. Vos, "Decision and Detection Limits for Linear Calibration Curves", Analytical Chemistry, Vol. 42. No. 8, pp. 849-855, July 1970
7. Standards of the National Environmental Laboratory Accreditation Conference, July 1999.
8. Guide to Method Flexibility and Approval of EPA Water Methods. U.S. Environmental Protection Agency Office of Water, 1996.
9. EPA Technical Support Document for the Assessment of Detection and Quantitation Approaches, February 2003, Chapter 2.