United States Environmental Protection Agency
Office of Water, Office of Wetlands, Oceans and Watersheds
Washington, D.C.
EPA 843-B-25-003

National Wetland Condition Assessment 2026

Laboratory Operations Manual

Version 1.1, March 2026

NOTICE

The intention of the National Wetland Condition Assessment (NWCA) 2026 project is to provide a comprehensive assessment forwetlands across the United States. The complete documentation of overall project management, design, methods, quality assurance, and standards is contained in four companion documents:

National Wetland Condition Assessment 2026: Field Operations Manual – 843-B-25-004
National Wetland Condition Assessment 2026: Quality Assurance Project Plan – 843-B-25-005
National Wetland Condition Assessment 2026: Laboratory Operations Manual – 843-B-25-003
National Wetland Condition Assessment 2026: Site Evaluation Guidelines – 843-B-25-006

This document (Laboratory Operations Manual) contains information on the methods for analyses of the samples to be collected during the project, quality assurance objectives, sample handling, and data reporting. Methods described in this document are to be used specifically in work relating to the NWCA 2026. All Project Cooperator laboratories must follow these guidelines. With the exception of the requirements in **Section 7.0** for evaluating microcystins, mention of trade names or commercial products in this document does not constitute a recommendation for use. **Section 7.0** requires use of a specific kit and supplemental materials manufactured by a single firm.

More details on specific methods for site evaluation, sampling, and sample processing in the field can be found in the appropriate companion document.

The suggested citation for this document is:

> USEPA. 2026. *National Wetland Condition Assessment 2026: Laboratory Operations Manual*. EPA-843-B-25-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

VERSION HISTORY

Version	Date	Changes Made
1.0	02/19/2026	Not Applicable
1.1	03/26/2026	Fixed Table of Contents links Correcting misspellings and font styles Corrected text referencing collection of five QA specimens

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

ASTM	American Society for Testing and Materials
Ca	calcium
CO_2	carbon dioxide
CV	coefficent of variation
DI	de-ionized
DL	detection limit
DOC	dissolved organic carbon
ELISA	enzyme-linked Immunosorbent assay
EPA	U.S. Environmental Protection Agency
FOM	Field Operations Manual
GDIT	General Dynamics Information Technology
GLEC	Great Lakes Environmental Center
GPS	global positioning system
HCl	hydrogen chloride
HGM	hydrogeomorphic
HDPE	high density polyethylene
HNO_3	nitric acid
HQ	headquarters
H_2SO_4	sulphuric acid
IM	information management
ISO	International Organization for Standardization
ITIS	Integrated Taxonomic Information System (ITIS)
K	potassium
KC	kit control
LIMS	Laboratory Information Management System
LOM	Lab Operations Manual
LRL	lower reporting limit
Mg	magnesium
MDL	method detection limit
MQO	measurement quality objective
MSDS	Materials Safety Data Sheet
N	nitrogen
Na	sodium
NARS	National Aquatic Resource Surveys
NC	negative control
ND	non-detect
NELAC	National Environmental Laboratory Accreditation Conference
NELAP	National Environmental Laboratory Accreditation Program
NH_4	ammonium
NIST	National Institute of Standards
NO_2	nitrite
NO_3	nitrate
NTU	Nephelometric Turbidity Units
NVC	National Vegetation Classification
NWCA	National Wetland Condition Assessment
OW	EPA Office of Water
PPT	Parts per thousand
PQL	practical quantification limit
PT	proficiency test
PTD	percent taxonomic disagreement

QA	quality assurance
QAPP	Quality Assurance Project Plan
QA/QC	quality assurance/quality control
QC	quality control
QCCS	quality control check solution
QMP	Quality Management Plan
QRG	Quick Reference Guide
RL	reporting limit
RO	reverse-osmosis
RSD	relative standard deviation
S	standard deviation
SEG	Site Evaluation Guidelines
SO_2	sulphur dioxide
SO_4	sulphate
SOPs	Standard Operating Procedures
SRM	standard reference material
TMB	tetramethylbenzidine
TN	total nitrogen
TP	total phosphorus
UNK	unknown
USGS	United States Geological Survey
UV	Ultraviolet

1.0 INTRODUCTION

The U.S. Environmental Protection Agency (EPA), in partnership with state and tribal organizations, has designed the National Wetland Condition Assessment (NWCA) 2026 to assess the condition of the nation's wetlands. The NWCA is one in a series of National Aquatic Resource Surveys (NARS) conducted to provide the public with a comprehensive assessment of the condition of the nation's waters. In addition to wetlands, NARS assesses coastal waters, lakes, rivers, and streams in a revolving sequence.

This manual, the NWCA Laboratory Operations Manual (LOM) contains procedures for laboratory analysis of samples collected from wetlands throughout the conterminous 48 states of the United States and Guam. The purposes of this manual are to:

1) document the standardized sample processing and analysis procedures used in the various laboratories for the NWCA 2026; and
2) provide guidance for data quality and a performance-based method approach to obtain comparable results across all participating laboratories.

Detailed laboratory procedures are described for the following indicators: vegetation, soils, water chemistry and chlorophyll-*a*, and microcystins. It should be noted that specific laboratory analysis procedures for water chemistry and chlorophyll-*a* samples are not presented here. A list of parameters to be analyzed as well as the performance-based methods and pertinent quality assurance/quality control (QA/QC) procedures are outlined as requirements for laboratories to follow when analyzing water chemistry and chlorophyll-*a* samples. Alternative analytical methods for water chemistry are acceptable if they meet all specified performance requirements described in this document. Acceptability is determined by the NWCA Headquarters (HQ) Project Management Team (EPA Office of Water). Please contact the NARS Laboratory Review Coordinator with any questions about method acceptability.

2.0 GENERAL LABORATORY GUIDELINES

This section describes the general laboratory guidelines with an overview to the QA/QC requirements. Each of the following sections describes analytical procedures, and the relevant QA/QC requirements, for a different indicator. In addition, the Quality Assurance Project Plan (QAPP) provides comprehensive descriptions of the QA/QC requirements for NWCA 2026.

2.1 Responsibility and Personnel Qualifications

All laboratory personnel shall be trained in advance in the use of equipment and procedures used for the standard operating procedure (SOP) in which they are responsible. All personnel shall be responsible for complying with all of the QA/QC requirements that pertain to the samples to be analyzed. Each lab should follow its institutional or organizational requirements for instrument maintenance. Specific lab qualification documentation required for analysis is contained in the QAPP.

2.2 Roles and Contact Information

Table 2.1 presents contact information for the key personnel associated with NWCA 2026. The **HQ Project Management Team** consists of the NWCA Project Manager, Project QA Coordinator, NARS Technical Advisor, EPA Logistics Coordinator, Laboratory Review Coordinator and the NARS Team Leader. The Team is responsible for overseeing all aspects of the project and ensuring technical and quality assurance (QA) requirements are properly carried out. The Team is the final authority on all decisions regarding laboratory analysis.

The **EPA Logistics Coordinator**, **Contractor Field Logistics Coordinator** and the **NARS Information Management (IM) Coordinator** track the location of each NWCA 2026 sample to be processed in the laboratory. The Contractor Field Logistics Coordinator and the NARS IM Coordinator are the laboratories' main point of contact in regard to sample tracking.

Table 2-1. Contact information. [1]

Title	Name	Contact Information
NWCA Project Manager	Gregg Serenbetz, OW	serenbetz.gregg@epa.gov; 202-566-1253
Project QA Coordinator	Sarah Lehmann, OW	Lehmann.sarah@epa.gov; 202-566-1379
Laboratory Review Coordinator	Kendra Forde, OW	kendra.forde@epa.gov; 202-566-0417
EPA Logistics Coordinator	Brian Hasty, OW	Hasty.Brian@epa.gov; 202-564-2236
NARS Technical Advisor	Amanda Nahlik, OW	Nahlik.amanda@ epa.gov; 541-754-4581
NARS Team Leader	Sarah Lehmann, OW	lehmann.sarah@epa.gov; 202-566-1379
NARS IM Coordinator	Michelle Gover, GDIT	gover.michelle@epa.gov ; 541-754-4793
Contractor Field Logistics Coordinator	Chris Turner, GLEC	cturner@glec.com; 715-829-3737

[1] For any technical direction, laboratories under contract to EPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the contacts provided in this table. For any technical information or sample tracking discussions, the laboratories are permitted to contact these people.

2.3 Laboratory Capability Review

Capability reviews are intended to familiarize EPA with actual procedures being implemented by different laboratories; and to ensure a clear and consistent understanding of procedures and activities by both EPA and the laboratories. After the initial remote evaluation of lab certifications and accreditations of all labs, the EPA may elect to conduct a lab capability review at any time during the period of performance of any lab task order and will provide no fewer than five working days' notice via written technical direction prior to the review. If EPA decides to conduct a capability review, a qualified EPA scientist or contractor will review a checklist based upon the steps described in this section. Lab capability reviews may be conducted virtually or in-person. EPA refers to in-person reviews as assistance visits for NWCA. EPA will develop, review, and approve the checklist and share the checklist with the lab prior to conducting a capability review.

2.4 Sample Tracking

Samples are collected by field crews during the index period (April through September). The actual number of wetlands sampled on a given day will vary widely during this time. Field crews will submit data electronically via the NWCA Field App or in rare instances, on paper forms. When sample information is submitted via the Field App, it will immediately update the NARS IM database. Field crews submit electronic forms when they have shipped samples and the NARS IM Center will input each sample into the NARS IM database. The NARS IM Coordinator provides laboratories with spreadsheets of samples that the batch laboratory or field crews (as appropriate) have sent to them. Upon sample receipt, the analysis laboratory must immediately complete and email the sample tracking spreadsheet (containing the sample login and sample condition information) to the NARS IM Coordinator for confirmation of sample receipt.

When the samples arrive from the field crews or batch laboratory, laboratories should also receive tracking forms in the shipment (refer to the NWCA 2026 FOM). These forms will list the samples that should be included in the shipment. Laboratory personnel must cross check the forms with the samples received to verify that there are not any inconsistencies. If any sample is missing or damaged, the laboratory must contact the NARS IM Coordinator and/or the Contractor Field Logistics Coordinator immediately. For States conducting analyses in their own laboratories, a state sample tracking spreadsheet is available from EPA.

2.5 Reporting

All laboratories must provide data analysis information to the HQ Project Management Team and the NARS IM Center by **December 15, 2026** or as stipulated in contractual agreements. These reports must include the following information and be reported in the data templates available separately from EPA:

- Sample Type (indicator)
- Site ID (ex: NWCA26-AL-10001)
- Sample ID (ex: 999000)
- Pertinent information to the indicator
- Metadata for all fields

See APPENDIX A: DATA REPORTING TEMPLATES for a list of reporting templates that laboratories will submit electronically. Electronic reporting templates will be provided on EPA's NARS SharePoint site.

The submitted file name must state the following:

- Indicator name (e.g., water chemistry)
- Date of files submission to NARS IM Center by year, month, and day (e.g., 2026_11_01)
- Lab name (e.g., MyLab)

Combined, the file name would look as follows: **WaterChemistry_2026_11_01_MyLab.xlsx**

Before the laboratory submits the batch data to EPA, the analyst who generated the data and an experienced data reviewer independently check and review the data, as follows.

- The analyst shall review the data to ensure that:
  - Sample preparation information is correct and complete;
  - Analysis information is correct and complete;
  - The appropriate method and standard operating procedures were followed;
  - Analytical results are correct and complete;
  - Appropriate QA codes are reported when necessary;
  - QC samples were within established control limits;
  - Blanks (where appropriate) were within the appropriate QC limits; and
  - Documentation is complete.

- The data reviewer shall review the data package to verify that:
  - Calibration data (where appropriate) are scientifically sound and appropriate;
  - QC samples were within established control limits;
  - Qualitative and quantitative results are correct; and
  - Documentation is complete.

Accompanying its data submission, the laboratory shall provide a short narrative that includes the following information:

- Project summary referencing the relevant QC identification number, total number of samples in the deliverable and their sample ID numbers, and the analytical methodology used for analysis;
- Discussion of any protocol deviations that may have occurred during sample testing;
- Discussion of QC questions or issues that were encountered and the corrective measures taken;
- Definitions of any laboratory QC codes used in the data;
- Summary and discussion of samples that are diluted by the presence of an interference, non-target analyte, or target analyte; and
- QC samples exceeding established control limits or parameters required by laboratory internal analytical SOPs and an explanation of why, if known.

As specified in the QAPP, remaining sample material and specimens must be maintained by laboratories as prescribed in contracts/grants or as directed by the NWCA 2026 Project Manager. Unless otherwise authorized by the Project Manager, the laboratory shall retain:

- The sample materials, including vials, for a minimum of three (3) years from the date the EPA publishes the NWCA 2026 datafiles on the public website. During this time, the laboratory shall maintain the materials at the temperature specified in its laboratory method. The laboratory shall periodically check the sample materials for degradation. Unless the Project Lead arranges

for transfer of sample materials to EPA, at the end of the retention period, the laboratory shall follow its internal protocols for disposal.

- Original records, including laboratory notebooks and raw data files (including logbooks, bench sheets, and instrument tracings), for a minimum of three (3) years from the date that EPA publishes the final report.

The Project Manager is responsible for maintaining the following:

- Deliverables from contractors and cooperators, including raw data, which are permanent as per EPA Record Schedule 1035.
- EPA's project records, which are under Schedule 1035, are permanent.

See Section 6 of the NWCA QAPP for additional information on laboratory QC and competency processes.

3.0 LABORATORY QUALITY CONTROL

As part of the NWCA 2026, field samples will be collected at each assessment site. These samples will be sent to laboratories cooperating in the assessment. To ensure quality, each Project Cooperator laboratory analyzing samples from the NWCA 2026 will receive an evaluation from an NWCA Lab Evaluator. All Project Cooperator laboratories will follow these guidelines.

No national program of accreditation for lab processing for most NWCA indicators currently exists. For this reason, a rigorous program of laboratory evaluation has been developed to support the NWCA 2026.

Given the large number of labs participating in the NWCA 2026, it is not feasible to perform an assistance visit[2] (AV) on each of these laboratories. An AV would include an on-site visit to the lab lasting at least a day. As a result, the HQ Project Management Team will conduct a remote evaluation of lab certifications and accreditations of all labs. This process is part of the laboratory capability review described in section 2.3. If issues arise from the remote evaluation that cannot be resolved remotely then an on-site visit to the lab will be performed. The HQ Project Management Team believes this approach meets the needs of this assessment and can ensure QC on data generated by the participating labs. General information is provided here, and more specifics are provided in **Section 3.1**.

Competency. To demonstrate its competency/expertise, the laboratory shall provide analyte specific information to EPA; or information specific to the relevant biological indicator. EPA will accept one or more of the following as a demonstration of competency (see Appendix D for more detail):

- Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.
- Documentation detailing the competency of the organization, including professional certifications for water-related analyses, membership in professional societies, and experience with analyses that are the same or similar to the requirements of this method.

QA/QC requirements

To demonstrate its competency in QA/QC procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), QAPPs, and applicable SOPs (See Appendix D for more information).

The person in charge of quality issues for each cooperating laboratory shall sign the NWCA QAPP Certification Page to demonstrate the organization's commitment to adhere to the requirements in the NWCA 2026 QAPP and LOM.

3.1 Remote Evaluation/Technical Assessment

Procedural review and assistance personnel are trained to the specific implementation and data collection methods detailed in this NWCA 2026 LOM. Laboratory evaluation reinforces the specific

[2] The evaluation of the labs is being considered an Assistance Visit rather than an audit because the evaluation is designed to provide guidance to the labs rather than an "inspection" as in a traditional audit.

techniques and procedures for both field and laboratory applications. A remote evaluation procedure has been developed for performing assessment of all labs.

Laboratory evaluation will be conducted prior to data analysis to ensure that specific laboratories are qualified and that techniques are implemented consistently across the multiple laboratories generating data for the program. Laboratory evaluation plans have been developed to ensure uniform interpretation and guidance in the procedural reviews.

The procedure being utilized involves requesting the laboratory to provide documentation of its policies and procedures. For the NWCA 2026 project, we have requested that each participating laboratory provide the following documentation:

- The laboratory's Quality Manual, QMP or similar document;
- SOPs for each analysis to be performed;
- Long-Term Method Detection Limits (LT-MDLs) for each instrument used;
- Demonstration of Capability for each analysis to be performed;
- A list of the laboratory's accreditations and certifications, if any; and
- Results from Proficiency Tests for each analyte to be analyzed under the NWCA project.

If a laboratory has clearly documented procedures for sample receiving, storage, preservation, preparation, analysis, and data reporting; has successfully analyzed Proficiency Test (PT) samples (if required by EPA, EPA will provide the PT samples); has a Quality Manual that thoroughly addresses laboratory quality including standard and sample preparation, record keeping and QA non-conformance; participates in a nationally recognized or state certification program; and has demonstrated ability to perform the testing for which program/project the assistance visit is intended, then the length of an on-site visit will be minimum, if not waived entirely. A final decision on the need for an actual on-site visit should be made after the evaluation of the documentation requested.

If a laboratory meets or exceeds all the major requirements and is deficient in an area that can be corrected remotely, suggestions will be offered, and the laboratory will be given an opportunity to correct the issue. A correction of the deficiency will then be verified remotely. The on-site visit should only be necessary if the laboratory fails to meet the major requirements and needs help or fails to produce the requested documentation.

All labs must sign the NWCA 2026 QAPP signature page. In addition, all labs must sign a Lab Signature Form (APPENDIX D: LABORATORY REMOTE EVALUATION FORMS) indicating that they will abide by the following:

- Utilize procedures identified in the NWCA 2026 LOM (or equivalent). If using equivalent procedures, please provide procedures manual to demonstrate ability to meet the required DQOs.
- Read and abide by the NWCA 2026 QAPP and LOM procedures/DQOs.
- Have an organized IT system in place for recording sample tracking and analysis data.
- Provide data using the template referenced in the LOM.
- Provide data results in a timely manner. This will vary with the type of analysis and the number of samples to be processed. Sample data must be received no later than December 15, 2026 or as otherwise negotiated with EPA.

- Participate in a lab technical assessment if requested by EPA NWCA staff (this may be a conference call or on-site assistance visit).

If a lab is participating in a biological analyses, they must, in addition, abide by the following:

- Use taxonomic standards outlined in the NWCA 2026 LOM.

4.0 VEGETATION

4.1 Introduction

Wetland plant species represent diverse adaptations, ecological tolerances, and life history strategies; and effectively integrate environmental conditions, species interactions, and human-caused disturbance. Data describing plant species composition and abundance, as well as vegetation structure are powerful, robust, and relatively easy to gather. They can be used to derive a myriad of metrics or indicators that are useful descriptors of ecological integrity or stress (e.g., Lopez and Fennessy 2002, USEPA 2002, Pino et al. 2005, Bourdaghs et al. 2006, Quétier et al. 2007, Magee et al. 2008, Magee et al. 2010, Mack and Kentula 2010). NWCA collects data on plant species composition and abundance, on vegetation structural attributes, and on ground surface attributes within vegetation plots at each sample site. The vegetation data are later used during analysis to calculate numerous metrics in a variety of categories that inform the development of Vegetation Multimetric Indices that serve as indicators of wetland vegetation condition (USEPA 2016, Magee et al. 2019a, b). Thus, the vegetation data collected in the field by the Vegetation Team are central to the key descriptors of ecological condition for the NWCA. The field data and metrics can also be used to characterize wetland vegetation across the NWCA target population or subpopulations.

4.1.1 Field Collection/Sample Summary

For NWCA, crews will collect unknown plant specimens ("unknown species vouchers") and a subset of known plant species for QA purposes ("QA vouchers") from each site and send them to a designated laboratory/herbarium for identification. To prepare QA vouchers and unknown species vouchers, field crews use a standard plant press to collect and arrange the specimens between newsprint and cardboard to preserve diagnostic features. Plant specimens are pressed and dried individually in labelled newsprint. Once dry, specimens (each in their respective newsprint) are placed into one or more labelled herbarium folders. Each folder label contains site information, folder number, and the number of specimens contained within the folder. Herbarium folders are placed in a provided zip-top bag and then into a plant shipping box with packing material as needed to prevent shifting.

4.2 Receiving Voucher Samples

Pressed plant samples will arrive at the vegetation laboratory/herbarium in shipping boxes containing labelled herbarium folders. Each plant sample should arrive with a plant specimen label (see **Section 4.4.1**).

4.2.1 Definitions

For the NWCA, a **voucher sample** is a pressed and dried plant sample, ideally comprised of leaves, stems, flowers, fruits and roots. An integral component of each voucher sample is written data describing the location, date of collection, habitat, plant habit, characteristic features, and other information. Vouchers provide physical evidence that confirms the presence of plant species at specific locations.

For all NWCA field work, whenever the identity of a species cannot be confirmed in the field, a sample is collected (see Vegetation Chapter of Field Operations Manual (FOM)) for later identification in the office or lab. All unknown species located in one of five Vegetation Plots in the Assessment Area (AA) that are mature and have key structures needed for identification are collected (**unknown species voucher**).

Unknown species that are immature or senescent comprising more than 5% cover are also collected. If an unknown species specimen is collected at a previous site, it is collected at subsequent sites, until the field botanist/ecologist learns the identity of the species and can reliably sight-recognize it in the field. This is particularly important for species in difficult-to-identify wetland genera and families, such as those that include sedges, rushes, grasses, and submerged aquatic vegetation. The field botanist/ecologist will transfer unknown samples to the **identifying botanist** at the laboratory/herbarium for initial identification.

For the purposes of this manual, the identifying botanist represents the person identifying and processing unknown samples. This could be the field botanist/ecologist; a university, state, national or regional herbarium botanist; or an EPA contractor that has qualifying credentials in plant taxonomy. The identifying botanist is responsible for ensuring all plant identification and processing tasks outlined in this manual are completed. In some cases, this may require lab partners to assist with the work.

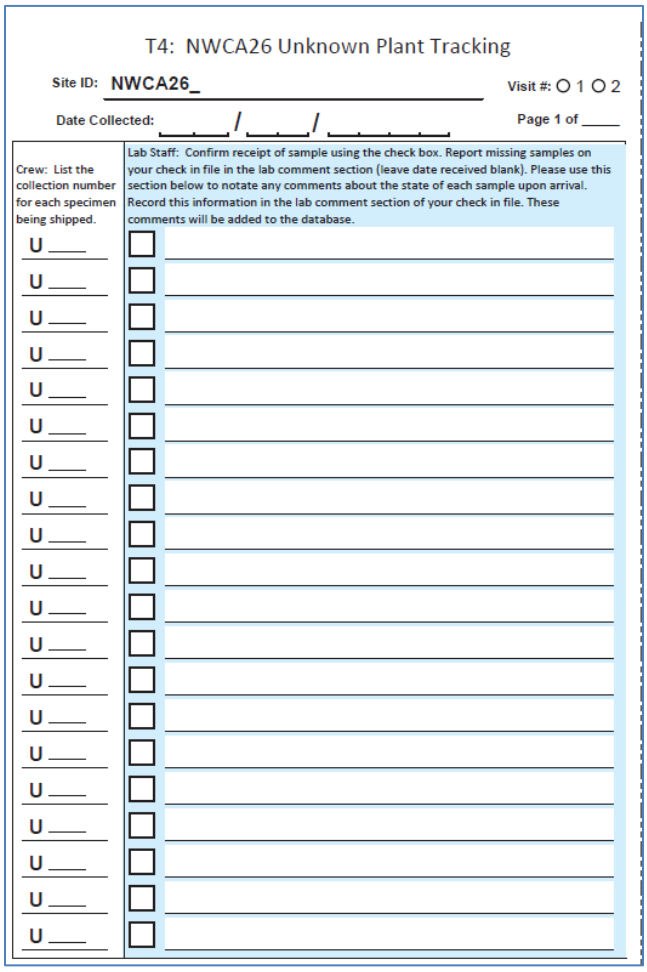
In addition to unknown specimens, field crews submit three known plant voucher samples, randomly selected from species identified by the Vegetation Team, for QA (QAPP Section 5.1.4). These **QA voucher** specimens are sent to a **QA verifying botanist** for re-identification/verification. Collecting voucher samples of known species both provides a quality check on species identity data, and a permanent record of the occurrence of a species at a given location.

The QA verifying botanist is responsible for re-identification/verification of the QA vouchers as well as a random selection of 10% of the unknown specimens that were initially determined by the identifying botanist in the lab.

If the unknown species specimens and QA voucher samples are planned to be sent to the same institution, it is important that the 10% re-identification/verification of unknown species are completed by a taxonomist that did not participate in the initial identification.

4.2.2 Tracking Information

In the field, each voucher sample collected is assigned a set of tracking information, which is recorded on the NWC26 Plant Tracking Forms (**Figure 4-1** and **Figure 4-2**). Plants are pressed at the end of the sample day and dried in a standard plant press inside sheets of folded newsprint. Once dry, the Vegetation Team will remove the samples and newsprint folders from the press, ensuring they retain the Plant Specimen Label (**Figure 4-3**), and transfer them in a sturdy box to either the **identifying botanist** for unknown samples or the **QA verifying botanist** for the three known specimens. If a sample listed on the tracking form is not part of the shipment or a sample arrives at the lab without the proper label, contact the HQ Project Management Team immediately.



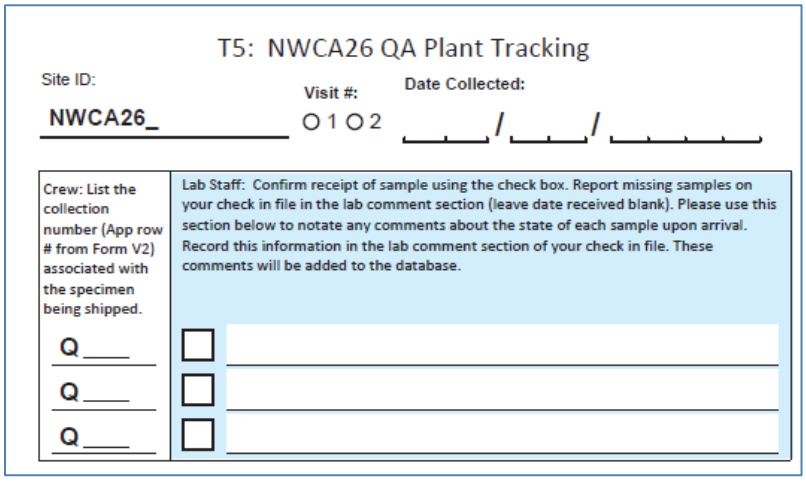
T4: NWCA26 Unknown Plant Tracking

Site ID: NWCA26_ Visit #: O 1 O 2

Date Collected: ____/____/_____ Page 1 of ____

Crew: List the collection number for each specimen being shipped.	Lab Staff: Confirm receipt of sample using the check box. Report missing samples on your check in file in the lab comment section (leave date received blank). Please use this section below to notate any comments about the state of each sample upon arrival. Record this information in the lab comment section of your check in file. These comments will be added to the database.
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐
U____	☐

Figure 4-1. Unknown plant sample tracking form.



T5: NWCA26 QA Plant Tracking

Site ID: NWCA26_ Visit #: O 1 O 2 Date Collected: ____/____/_____

Crew: List the collection number (App row # from Form V2) associated with the specimen being shipped.	Lab Staff: Confirm receipt of sample using the check box. Report missing samples on your check in file in the lab comment section (leave date received blank). Please use this section below to notate any comments about the state of each sample upon arrival. Record this information in the lab comment section of your check in file. These comments will be added to the database.
Q____	☐
Q____	☐
Q____	☐

Figure 4-2. QA plant sample tracking form.

4.3 Supplies and Equipment for Sample Handling

- Plant dryer
- Dissecting microscope
- Herbarium cabinet or sealable plastic boxes for storing dried plant samples prior to identification
- Dissecting tools (e.g., single edge razor blades, forceps, dissecting needles)
- Regional floras and plant lists
- Access to USDA-NRCS PLANTS standardized taxonomic nomenclature (https://plants.usda.gov/)
- 2026_NWCA Plant ID_Lab Spreadsheets.xlsx data template
- Plant sample tracking forms
- Plant sample folders
- Freezer or other laboratory approved treatment supplies for killing pests on dried plant material
- OPTIONAL: Mounting materials (herbarium sheets, mounting glue, forceps, weights for holding samples with wet glue to the herbarium sheets, etc.)
- OPTIONAL: Herbarium sample labels

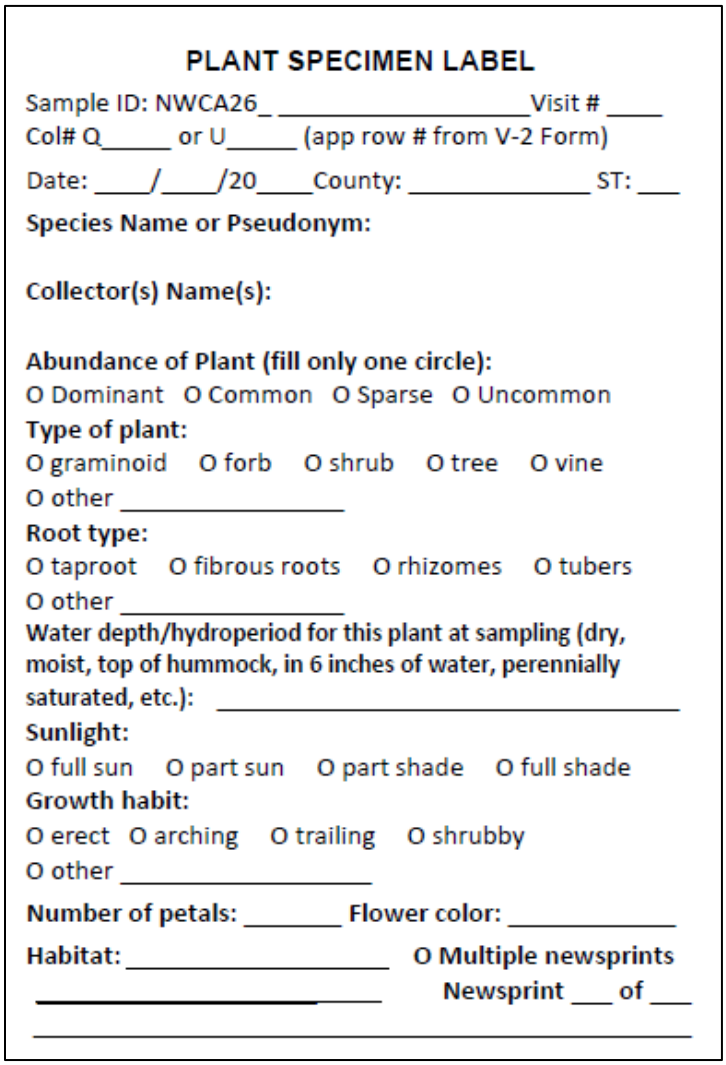
4.4 Handling Vegetation Samples

Plant samples may arrive at the vegetation laboratory/herbarium in the following conditions: 1) as dried, pressed samples, or 2) pressed but still wet plant material enclosed in a plant press.

1. If samples are pressed and dried, proceed to **Section 4.4.3** (Treat samples for detritivores, molds, and pests).
2. If samples arrive in a press but are still wet, they should be placed on a plant dryer to complete drying, and then be treated for detritivores, molds, or pests.

4.4.1 Plant Sample Label Form

Every plant sample sent to the vegetation laboratory/herbarium will be labeled with a plant specimen label. This label includes the original identification and key descriptive information that can aid in identifying an unknown species voucher or in verifying the identification of a QA voucher when reviewed at the vegetation lab/herbarium. Unknown species voucher samples are considered incomplete without this information. If the plant specimen label is missing information, contact the HQ Project Management Team immediately. An example of the plant specimen label is provided in **Figure 4-3** and descriptions for each label item are included in **APPENDIX C: SUPPLEMENTARY MATERIAL FOR VEGETATION - PLANT PRESSING AND MOUNTING**.



PLANT SPECIMEN LABEL

Sample ID: NWCA26_ _____Visit # ____
Col# Q_____ or U_____ (app row # from V-2 Form)

Date: ____/____/20____County: _____ ST: ___

Species Name or Pseudonym:

Collector(s) Name(s):

Abundance of Plant (fill only one circle):
O Dominant O Common O Sparse O Uncommon

Type of plant:
O graminoid O forb O shrub O tree O vine
O other _____

Root type:
O taproot O fibrous roots O rhizomes O tubers
O other _____

Water depth/hydroperiod for this plant at sampling (dry, moist, top of hummock, in 6 inches of water, perennially saturated, etc.): _____

Sunlight:
O full sun O part sun O part shade O full shade

Growth habit:
O erect O arching O trailing O shrubby
O other _____

Number of petals: _____ **Flower color:** _____

Habitat: _____ **O Multiple newsprints**
_____ **Newsprint ___ of ___**
_____

Figure 4-3. Plant specimen label.

4.4.2 Drying Samples

If a plant sample arrives wet in the plant press, it must be thoroughly dried before it is removed from the press. As a sample dries, it will lose volume, so it is often necessary to periodically tighten the straps on the press to maintain pressure on the sample and minimize shrinkage and wrinkling.

Low ambient humidity and good airflow around and through the press is important for rapid and thorough drying of plant material. Rapid drying over low heat promotes preservation of color and morphology resulting in high quality samples. Dry air circulating through the press also may kill many insects and insect eggs, which may protect the samples from insect damage. These conditions are most easily obtained by placing full presses on an electric plant dryer that provides steady bottom heat (95°F to 113°F), where plants usually dry in 12 to 48 hours. However, presses placed in a warm, dry place will be sufficient if a plant dryer is not available.

4.4.3 Treat Samples for Detritivores, Molds, and Pests

Dried plant material is highly susceptible to contamination by detritivores, molds, and pests that can destroy herbaria collections. Therefore, it is important to treat all incoming samples to kill potential contaminates.

Standard pest procedures of the herbaria should be implemented. A common method for sample treatment is to freeze treat them (at -20°C or below) for at least three days for loosely stacked samples and seven days for tightly stacked samples.

To protect the collection from pest infestation, plant samples should be stored in herbarium cabinets or sealable plastic containers when not in use. **Under no circumstances** should samples be left out overnight. If samples are found to have been left out overnight or if an herbarium cabinet/plastic container has been left open, all samples must be inspected and decontaminated again if pests are found.

4.5 Identification of Vegetation Samples

4.5.1 Taxonomic Standard

The recognition and identification of particular classes of plants such as families, genera, and species are a critical and difficult element of collecting accurate vegetation plot data. To complicate matters, not all botanical authorities agree about which name to apply to a particular plant species. The NWCA uses standardized nomenclature cited in the USDA-NRCS PLANTS Database (https://plants.usda.gov/) as its taxonomic standard. To effectively identify plants in the field, however, field crews may use local floras and plant keys appropriate to each region or state (APPENDIX B: SUPPLEMENTARY MATERIAL FOR VEGETATION – LISTS OF FLORISTIC RESOURCES). This means numerous taxonomies will likely be applied across the 48 conterminous states and Guam comprising the study area. The **identifying botanist** at the vegetation laboratory/herbarium will reconcile all species names to the standard found in USDA-NRCS PLANTS Database (https://plants.usda.gov/).

4.5.2 Recording Identifications

All identifications are recorded in an Excel data template (2026_NWCA Plant ID_Lab Spreadsheets.xlsx). The Excel data template includes user information tabs that provide quick reference lists and instructions for recording data. For example, a list of growth habit codes as well as floras or field guides are included for quick reference while other tabs provide examples and specific instructions on how to fill out the various data fields of the QA vouchers and unknown species vouchers spreadsheets. Once the spreadsheets in the data template have been completed, copies are sent to the EPA Project Management Team or their delegates (QAPP Section 5.1.5).

4.6 Mounting and Storing Herbarium Sheets

Once the samples are dried, pressed, and identified, they are to be stored at the vegetation laboratory/herbarium for at least five years. Vouchers should be kept in sealable plastic containers in a cool dry climate and must be accessible to the EPA. However, the vegetation laboratory/herbarium is encouraged to incorporate the NWCA vouchers into their permanent collections as desired. Vouchers from the national survey mounted on herbarium sheets should be labeled to indicate that they were collected as part of the NWCA. For an example of commonly used mounting and labeling methods see **APPENDIX C: SUPPLEMENTARY MATERIAL FOR VEGETATION - PLANT PRESSING AND MOUNTING.**

4.7 Quality Assurance

A subset of plant samples collected as unknown species vouchers and later identified by a State or National Plant Laboratory botanist ("identifying botanist") will be verified by a QA taxonomist ("verifying botanist") for additional QA. The lab will randomly select 10% of the identified unknown species vouchers for re-identification by another experienced taxonomist who did not participate in the original identifications. The NWCA QA Team will evaluate differences in the taxonomic identification of plant specimens between the identifying and verifying botanists. Substantial disagreements between the two botanists will be investigated and logged for indication of error patterns or trends, but all values will generally be considered acceptable for further analysis, unless the investigation reveals significant problems.

QC procedures associated with sample handling and processing at laboratories handling NWCA QA and unknown species vouchers are summarized in **Table 4-1**.

Table 4-1. Vegetation indicator: laboratory QC activities.

QC Activity	Frequency	Acceptance Criteria	Corrective Action
Demonstrate competency for identifying plant specimens to meet the performance measures	Once	Demonstration of past experience relevant to identifying plants collected from wetlands	EPA will not approve any lab for NWCA voucher identifications that cannot demonstrate competency. In other words, EPA will select another lab that can demonstrate competency.
Verify that plant voucher has arrived in acceptable condition	All vouchers	The condition must allow for positive identification	Lab will consult immediately with EPA TOCOR if voucher does not arrive in acceptable condition.
Voucher log-in	All vouchers	Plant vouchers logged into NARS IM system within 24 hours of receipt	Discrepancies, damaged or missing vouchers, and missing plant specimen label information are reported to NWCA Project Manager and Laboratory Review Coordinator.
Store vouchers appropriately	All vouchers	Vouchers must be treated to kill potential contaminants and properly stored dry in a condition that prevents contamination by detritivores, molds, and pests (typically in herbarium cabinets or sealable plastic containers)	EPA will not approve any laboratory that cannot demonstrate appropriate storage of vouchers. In other words, EPA will select another lab that can demonstrate appropriate voucher storage.

QC Activity	Frequency	Acceptance Criteria	Corrective Action
Use widely and commonly accepted taxonomic references and reconcile to USDA-NRCS PLANTS standardized taxonomic nomenclature	All identifications	Full citations for floras and field guides used in plant identification must be provided; identifications must be reconciled to the standardized taxonomic nomenclature of the USDA-NRCS PLANTS database	Lab will provide explanation and discuss deviances with EPA TOCOR.
QA voucher identification by lab/herbarium	All QA vouchers	Identification by the lab's QA verifying botanist	Disagreement with field botanist/ecologist identification reported to EPA TOCOR.
Unknown species voucher identification by lab/herbarium	All unknown species vouchers	Identification by the lab's identifying botanist	Replace field crew's "unknown" identification with determination by lab; if identification not possible, record comment with explanation (e.g. lack of distinguishing features, specimen condition).
Unknown species voucher QC	Approximately 10% of all unknown species vouchers independently identified in the lab	PTD ≤ 15%	If PTD > 15%, review data for possible explanations; otherwise, insert data qualifier for lab identifications.
Conduct AV	EPA may choose to visit any lab to conduct an AV	Performance must meet the requirements on AV checklist	Performance and any recommended improvements described in debrief with lab staff.

4.7.1 Percent taxonomic disagreement (PTD)

PTD is a measure of taxonomic precision comparing the number of agreements (positive comparisons, $comp_{pos}$) of the first plant ID specialist ("identifying botanist") and the second plant ID specialist ("verifying botanist") for unknown vouchers. In the following equation, *N* is the total number of specimens in the larger of the two counts. PTD should be ≤15%.

$$PTD = \left[1 - \frac{comp_{pos}}{N}\right] \times 100$$

The NWCA QA Team will monitor differences in the taxonomic identification of plant specimens between the identifying botanists providing the initial identification and the verifying botanists providing the independent re-identifications. Substantial disagreements between the two will be investigated and reasons for the discrepancies examined and corrected.

4.8 References

Kansas State University. 2006. Integrated Pest Management Plan.

Lee, M. T., R. K. Peet, S. D. Roberts, and T. R. Wentworth. 2008. CVS-EEP protocol for recording vegetation: All levels of plot sampling. Version 2008. The Carolina Vegetation Survey and the North Carolina Ecosystem Enhancement Program.

Mack, J. J. 2007. Integrated Wetland Assessment Program. Part 9: Field Manual for the Vegetation Index of Biotic Integrity for Wetlands v. 1.4. Ohio EPA Technical Report WET/2004-9. Ohio Environmental Protection Agency, Wetland Ecology Group, Division of Surface Water, Columbus, Ohio.

Magee, T. K., S. E. Gwin, R. G. Gibson, C. C. Holland, J. E. Honea, P. W. Shaffer, J. C. Sifneos, and M. E. Kentula. 1993. Research Plan and Methods manual for the Oregon Wetlands Study. EPA/600/R-93/072. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, Oregon. Stribling, J.B., S.R. Moulton, and G.T. Lester. 2003. Determining the quality of taxonomic data. Journal of the North American Benthological Society 22:621–631.

Magee, T.K., Blocksom, K.A. & Fennessy, M.S. 2019a. A national-scale vegetation multimetric index (VMMI) as an indicator of wetland condition across the conterminous United States. Environ Monit Assess 191, 322.

Magee, T.K., Blocksom, K.A., Herlihy, A.T. et al. 2019b. Characterizing nonnative plants in wetlands across the conterminous United States. Environ Monit Assess 191, 344.

University of Florida Herbarium. 2009. Preparation of Plant Samples for Deposit as Herbarium Vouchers. http://www.flmnh.ufl.edu/herbarium/voucher.htm, Accessed June 12, 2009.

USEPA. 2002. Methods for Evaluating Wetland Condition: #10 Using Vegetation to Assess Environmental Conditions in Wetlands. EPA-822-R-02-020, Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2011. National Wetland Condition Assessment. Laboratory Operations Manual. EPA843-R10-002. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2011. National Wetland Condition Assessment. Quality Assurance Project Plan. EPA843-R10-003. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2016. National Wetland Condition Assessment: 2011 Technical Report. EPA-843-R-15-006. US Environmental Protection Agency, Washington, DC.

5.0 SOILS

5.1 Introduction

Soils play an important role in wetland ecosystems, cycling nutrients, regulating water movement and storage, and serving as a growth medium or habitat for plants, microbes, and macroinvertebrates. Wetland soils develop distinct characteristics as a result of the hydrology and biota (e.g., microbes, vegetation) associated with wetlands, as well as other factors that influence soil development across all environments (e.g., climate, geology). These characteristics impact the functions and processes occurring in the soil and reflect ecological condition.

Field Collection/Sample Summary: Three types of soil samples are collected at each sampling site from a single Soil Plot within the Assessment Area. Six samples collected from the top 10 cm of the soil using a steel cylinder are combined into one plastic sample bag for the Standard Depth Soil Core (SDSC) sample and analyzed for chemical parameters and particle size distribution (soil texture). A Soil Horizon Bulk Density (SHBD) sample is collected from each soil horizon greater than 8 cm thick to a depth of 125 cm using a steel cylinder and placed into a plastic sample bag for analysis of bulk density. A Soil Horizon Chemistry (SHCH) sample is collected from each soil horizon to a depth of 125 cm and placed into a plastic sample bag for analysis of chemical parameters and particle size distribution (soil texture). All samples are stored at room temperature until shipment by the field crews to the NWCA national laboratory.

This section describes the methods for analyzing chemical and physical properties of soil samples collected in the NWCA.

5.2 Summary of Method

The SDSC, SHBD, and SHCH samples will be analyzed by the Great Lakes Environmental Center and their subcontractor. Multiple analyses will be conducted to characterize soil chemical and physical properties (**Table 5-1**). Soil bulk density measurements will be made on SHBD samples; all other parameters will be measured on the SDSC and the SHCH samples.

Analytical methods used by the laboratory shall be comparable to the methods described in the Kellogg Soil Survey Laboratory (KSSL) methods manual (Soil Survey Staff, 2022).

Table 5-1. Summary of NWCA 2026 soil analytical methods.

Analyte	Method	Summary of Method	KSSL Method or equivalent
Clay **Silt** **Sand**	PSDA, <2 mm, air dry	Organic matter removed; sand fraction removed by wet sieving; clay and fine silt fractions determined by pipetting following sedimentation; coarse silt is the difference between 100% and the sum of sand, clay, and fine silt.	3A1a1a
$CaCO_3$	Calcium Carbonate Equivalent, <2mm	Samples are treated with HCl; evolved CO_2 is measured manometrically; carbonate in the soil is calculated as percent $CaCO_3$.	4E1a1a1a1
	Calcium Carbonate Equivalent, 2 - 20 mm		4E1a1a1a2

Analyte	Method	Summary of Method	KSSL Method or equivalent
C **N** **S**	Total Carbon, Nitrogen, and Sulfur	Total Carbon, Nitrogen, and Sulfur are measured by dry combustion; released measuring components (N_2, CO_2, and SO_2) are measured using an elemental analyzer.	4H2a1-3
pH	1:1 H_2O	The pH is measured in soil-water (1:1) and soil-salt (1:2 $CaCl_2$) solutions using a combination pH-reference electrode.	4C1a2a1a-b1
	1:2 0.01 M $CaCl_2$		4C1a2a2a-b1
CEC **Ca^{2+}** **K^+** **Mg^{2+}** **Na^+**	Cation Exchange Capacity by NH_4OAc, pH 7	The CEC and base cations are determined by a displacement procedure. Sample is leached using 1 N NH_4OAc. Exchange sites are saturated by an index cation (NH_4^+) adsorbed by the soil, and soil is washed free of excess saturated salt. The index cation is displaced by rinsing with KCl and the leachate is analyzed by steam distillation and titration to determine the NH_4^+ adsorbed on the soil exchange complex. The NH_4OAc extract is diluted with an ionization suppressant (La_2O_3) and analytes (Ca^{2+}, K^+, Mg^{2+}, and Na^+) are measured by an inductively coupled plasma optical emission spectrophotometer (ICP-OES) or an inductively coupled plasma mass spectrometer (ICP-MS).	4B1a1a1a1 4B1a1b1-4
EC	Electrical Conductivity	Soil sample is mixed with water and allowed to stand overnight; electrical conductivity (EC) of the mixture is measured using an electronic bridge. The EC by this method is used to indicate the presence of soluble salts.	4F1a1a1a1
P (Olsen)	Olsen Extraction	This extractant is most applicable to neutral to calcareous soils (Buurman et al., 1996). Soil sample is shaken with Olsen sodium-bicarbonate extracting solution at pH 8.5, centrifuged, and filtered; clear extract is diluted with a color reagent; absorbance of the solution is read using a spectrophotometer at 882 nm.	4D5a1a-b1
P (Mehlich No. 3)	Mehlich No. 3 Extraction	Mehlich No. 3 is used as an index of available P in the soil. Extraction of P by Mehlich No. 3 is designed to be applicable across a wide range of soil properties with reaction ranging from acid to basic (Mehlich, 1984), and correlates with Olsen extractant on calcareous soils (R^2=0.918), even though the quantity of Mehlich No. 3 extractable P is considerably higher (Soil and Plant Analysis Council, 1999). Soil sample is shaken with Mehlich No. 3 extracting solution, centrifuged, and filtered; clear extract is diluted with a working solution; absorbance of the solution is read using a spectrophotometer at 882 nm.	4D6a1a-b1
Ag **Cd** **Co** **Cr** **Cu** **Ni**	Trace Elements	Microwave digestion methodology utilizing HNO_3 and HCl. Analyte concentrations are determined using an inductively coupled plasma mass spectrometer (ICP-MS). This method follows EPA Method 3051A.	4H1a1a1a1-20

Analyte	Method	Summary of Method	KSSL Method or equivalent
P **Pb** **Sb** **Sn** **V** **W** **Zn**			
Bulk Density	Bulk Density Core Method	Bulk density was determined for field-moist soil cores of known volume. The field-state bulk density (Dbf) value is the bulk density of a soil sample including the water content of the soil in the field at the time of sampling. A metal cylinder is pressed or driven into the soil; the cylinder is removed, extracting a sample of known volume. The moist sample weight is recorded, sample is dried in an oven and weighed. Db_f is the oven dry weight of the soil divided by the core volume and corrected for rock fragments (if present).	3B6a

5.3 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions.

In addition to understanding the laboratory's hazard communication, safety and disposal requirements, persons using this procedure must abide by the following health and safety procedures:

1. Laboratory facilities must properly store and dispose of solutions of weak acid.

2. Laboratory personnel must wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves).

3. When working with potentially hazardous chemicals (e.g., weak acid), laboratory personnel must avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin and mucous membranes with acid. If skin contact occurs, remove clothing immediately. Wash and rinse the affected skin areas thoroughly with large amounts of water.

5.4 General Requirements

Expertise. To demonstrate its competency/expertise to address each of the applicable parameters, the laboratory shall provide EPA with performance data demonstrating their proficiencies in analyzing water quality samples. See APPENDIX D: LABORATORY REMOTE EVALUATION FORMS for more information.

QA/QC requirements. To demonstrate its expertise in QA/QC procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include QMPs,

QAPPs, and applicable SOPs. See APPENDIX D: LABORATORY REMOTE EVALUATION FORMS for more information.

To demonstrate its ongoing commitment to adhere to the requirements in the NWCA 2026 QAPP and LOM, the person(s) in charge of quality issues for the organization shall sign the NWCA QAPP Certification Page.

5.5 Sample Handling and Processing

5.5.1 Receiving Regulated Soils

Soils that may contain pests (i.e., bacteria, plant viruses, fungi, nematodes, and life stages of destructive mollusks, acari, and insects) are regulated by U.S Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). Areas within states that are under Federal quarantine must follow the conditions and safeguards prescribed by APHIS before shipping to another part of the country. To ensure that the NWCA is in compliance with APHIS recommendations, all soils collected for the survey will be shipped as regulated soils. Participating labs are responsible for obtaining and maintaining a valid permit for receiving regulated soils.

Upon arrival at the lab, soil samples will be separated into regulated and non-regulated based on their county and state of origin (as recorded on the waterproof label affixed to the outside of the sample bag). The lab is responsible for following all APHIS protocols when handling or disposing regulated soils as found in 7 CFR 330.300.

5.5.2 Sample Receipt

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery. For each sampled site, the lab will receive the following samples:

- One bag of soil, approximately 1 liter, labeled 'SDSC' for soil chemistry analysis
- One or more bags of soil, approximately 1 liter each, labeled 'SHCH' (depending on the number of horizons collected) for soil chemistry analysis
- One or more bags of soil, approximately 1 liter each, labeled 'SHBD' (depending on the number of horizons collected) for bulk density analysis

The laboratory technician must inspect the samples promptly on receipt and enter information onto a spreadsheet provided by the NARS IM Coordinator to log the samples into the NARS IM system within 24 clock hours. Steps in this process include:

1. Checking that each shipping container has arrived undamaged, and recording the condition of the sample on the sample tracking spreadsheet using the codes in
2. Verifying that all required data elements, per **Table 5-2,** have been recorded in the tracking spreadsheet. If any data elements are missing, then enter them into the spreadsheet before submitting to NARS IM.

Additionally, the laboratory technician must notify the EPA immediately about any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.

Table 5-2. Soil samples login: required data elements.

Variable	Type	Description	
SITE_ID	Character	Site identification code	
SAMPLE_ID	Character	Sample number	
DATE_COL	Date	Date that the field crew collected the sample	
DATE_RECEIVED	Date	Date that the sample was received	
SAMPLE_TYPE	Character	Standard depth soil core (SDSC), horizon chemistry (SHCH) or horizon bulk density (SHBD)	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		VT	Volume is not sufficient for testing
		VR	Volume is not sufficient for a retest, if required
		Q	Other quality concerns, not identified above
CONDITION_COMMENT	Character	Explanation for Q FLAG (if needed)	

5.5.3 Sample Preparation

For most standard chemical, physical, and mineralogical analysis, the field sample is air-dried, crushed, and sieved to <2 mm. The protocol for preparing soil samples and descriptions of preparation methods for specific analyses are given in the Kellogg Soil Survey Laboratory (KSSL) methods manual (Soil Survey Staff, 2022).

5.6 Quality Measures

This section describes the QA/QC measures used to ensure that the data will meet NWCA's requirements. Standardized protocols, consistent training of all technicians, and availability of experienced technical personnel to respond to site-specific questions as they arise are important to ensuring the quality of the data. Additionally, control measures to minimize measurement error among laboratory technicians and laboratories include the use of laboratory QC samples and a data review and validation process (QAPP Section 5.2.5).

5.6.1 Laboratory Performance Requirements

Laboratory performance requirements for the soil indicators are summarized in **Table 5-3**.

Table 5-3. Soil indicator: laboratory method performance requirements.

Type	Parameter	Units	MDL	EPA Targets Accuracy	Precision
Particle Size (PSDA)	Clay	%	0.05	10%	15%
	Silt	%	0.05	10%	15%
	Sand	%	0.05	10%	15%
Calcium Carbonate Equivalent	CaCO3 (<2mm)	%	0.5	10%	15%
	CaCO3 (2-20mm)	%	0.5	10%	15%
Total Carbon, Nitrogen, Sulfur	Carbon	%	0.04	10%	15%
	Nitrogen	%	0.04	10%	15%
	Sulfur	%	0.04	10%	15%
pH	1:1 H2O	n/a	n/a	10%	15%
	1:2 0.01 M CaCl2	n/a	n/a	10%	15%
Cation Exchange Capacity	CEC	cmol (+) kg^{-1}	0.1	10%	15%
	Ca2+	cmol (+) kg^{-1}	0.07	10%	15%
	K+	cmol (+) kg^{-1}	0.06	10%	15%
	Mg2+	cmol (+) kg^{-1}	0.01	10%	15%
	Na+	cmol (+) kg^{-1}	0.2	10%	15%
Electrical Conductivity	EC	mmhos/cm	0.001	10%	15%
Phosphorus	P (Mehlich No. 3)	mg/kg	0.1	10%	15%
	P (Olsen)	mg/kg	0.1	10%	15%
Bulk Density Core Method	Bulk Density	g/cm3	na	10%	15%
Trace Elements	Antimony	mg/kg	0.02	10%	15%
	Cadmium	mg/kg	0.01	10%	15%
	Chromium	mg/kg	0.01	10%	15%
	Cobalt	mg/kg		10%	15%
	Copper	mg/kg	0.02	10%	15%
	Lead	mg/kg	0.01	10%	15%
	Nickel	mg/kg	0.01	10%	15%
	Phosphorus	mg/kg	0.4	10%	15%
	Silver	mg/kg	0.01	10%	15%
	Tin	mg/kg	0.05	10%	15%
	Tungsten	mg/kg		10%	15%
	Vanadium	mg/kg		10%	15%
	Zinc	mg/kg	0.01	10%	15%

5.6.2 Laboratory Quality Control Samples

Laboratory QC samples for the soil indicators include control samples and blank samples.

A control sample represents a sample of known concentration for a particular attribute. A control sample is collected in bulk for an attribute and repetitively analyzed to determine statistical control limits (i.e., range of expected values) for the particular method. A control sample is analyzed in conjunction with every batch of samples to ensure the method was run correctly. If the value of the control sample falls outside the expected range of values, then the process has failed and the batch is flagged for reanalysis.

A blank sample is used to ensure equipment is thoroughly cleaned before each use. A blank sample is especially important when measuring soil chemistry (i.e., trace metals) because concentrations may be quite small. A blank sample is analyzed in conjunction with every batch of samples to ensure that proper equipment cleaning protocols are followed. If the value of the blank sample does not equal zero or falls below the method detection limit, then the equipment is not clean and the batch is flagged for reanalysis.

5.6.3 Data Reporting, Review, and Management

The data validation process involves multiple data reviews to:

- verify that blank and control samples return results that fall within established control limits;
- examine the data for inconsistencies and apparent anomalies; inconsistencies usually take the form of unexpected high or low values for a particular analyte or values that do not fit with the expected trend of a soil profile;
- determine whether the project data are self-consistent and congruent with the site data collected in the field; incongruities within the data that can be explained either by site data or the results of other analytes are recorded; and
- conduct range checks, summary statistics, and/or exploratory data analysis.

Identified reporting errors are corrected or data is qualified as suspect or invalid as appropriate and the laboratory QC coordinator determines impact and possible limitations on overall usability of data based on the specific issue. The NWCA 2026 Project QA Coordinator is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members. A summary of the QC requirements are provided in Table 5-4.

Table 5-4. QC activities for soil samples.

QC Requirement	Description	Frequency	Acceptance Criteria	Corrective Action
Demonstrate competency for analyzing soil samples to meet the performance measures	Demonstration of past experience with soil samples in achieving the method detection/reporting limits.	Once	See APPENDIX D: LABORATORY REMOTE EVALUATION FORMS	EPA will not approve any laboratory for NWCA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can demonstrate competency for its NWCA samples.
Check condition of sample when it arrives.	Sample issues such as cracked container(s); missing label(s); sufficient volume for test.	Once	No sample issues or determination that sample can still be analyzed	Lab determines if the sample can be analyzed or has been too severely compromised (e.g., contamination). Assign appropriate condition code identified in Table 5-2.
Store sample appropriately.	Samples stored according to laboratory's SOPs.	Weekly or per laboratory's SOPs	No deviations from normal storage SOPs.	EPA expects that the laboratory will exercise every effort to maintain samples properly.
Control sample verification	Control samples return results that fall within established control limits	Once per analytical batch	Within expected range of values	Batch is flagged for reanalysis.
Blank sample verification	Blank samples return results that fall within established control limits	Once per analytical batch	Value of the blank equals zero	Batch is flagged for reanalysis.
Data inconsistency	Unexpected high or low values for analyte outside expected trend of soil profile; analyte result not congruent with other site data	Data reporting	Within expected range	Reporting errors corrected or qualified as suspect or invalid as appropriate.
Use consistent units for QC samples and field samples	Verify that all units are provided consistently within each indicator.	Data reporting	For each indicator, all field and QC samples are reported with the same measurement units	If it is not possible to provide the results in consistent units, then assign a QC code and describe the reason for different units in the comments field of the database.

5.7 Sample and Record Retention

The laboratory shall retain:

- The sample materials until the date EPA publishes the NWCA 2026 data publicly (not including provisional or draft publication) or the contractor is notified to dispose of the materials in

written technical direction from the TOCOR. During this time, the laboratory shall maintain the materials as specified in its laboratory method. The laboratory shall periodically check the materials for degradation..

- Original records, including laboratory notebooks for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

5.8 References

Buurman, P., B. van Lagen, and E.J. Velthorst. 1996. Manual for soil and water analysis. Backhuys Publ., Leiden, the Netherlands.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis 15:1409-1416.

Soil and Plant Analysis Council, Inc. 1999. Handbook on Reference Methods for Soil Analysis. Council on Soil Testing and Plant Analysis. CRC Press, Boca Raton, FL.

Soil Survey Staff. 2022. Kellogg Soil Survey Laboratory methods manual. Soil Survey Investigations Report No. 42, Version 6.0. U.S. Department of Agriculture, Natural Resources Conservation Service.

USEPA. 2011. National Wetland Condition Assessment. Laboratory Operations Manual. EPA843-R10-002. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2011. National Wetland Condition Assessment. Quality Assurance Project Plan. EPA843-R10-003. U.S. Environmental Protection Agency, Washington, DC.

6.0 WATER CHEMISTRY AND CHLOROPHYLL-A

6.1 Introduction

This section describes the analysis requirements for water quality samples. The purpose is to determine concentrations of water quality parameters in samples collected in the NWCA 2026. The laboratory shall perform analyses to determine levels of conductivity, pH, ammonia (NH_3), nitrate-nitrite (NO_3-NO_2), total nitrogen (TN), total phosphorus (TP), turbidity, dissolved organic carbon (DOC) and chlorophyll-*a* found in freshwater and saline wetlands, and sulfate (SO_4) and chloride (Cl) for freshwater samples only (**Table 6-1)**.

Table 6-1. Water chemistry parameters measured by NWCA 2026.

Analyte	Units	Comments
Conductivity	µS/cm at 25˚C	All samples
pH (laboratory)	Standard (Std) Units	All samples
Turbidity	Nephelometric Turbidity Units (NTU)	All samples
Dissolved Organic Carbon (DOC)	mg C/L	All samples
Ammonia (NH_3)	mg N/L	All samples
Nitrate-Nitrite (NO_3-NO_2)	mg N/L	All samples
Total Nitrogen (TN)	mg/L	All samples
Total Phosphorus (TP)	µg P/L	All samples
Sulfate (SO_4)	mg SO_4/L	Freshwater samples only
Chloride (Cl)	mg Cl/L	Freshwater samples only
Chlorophyll-*a*	µg/L (in extract)	All samples

Field Collection/Sample Summary: Crews collect samples for water chemistry analyses (CHEM) in 1-liter cubitainers. Crews collect water in a 1 L amber HDPE bottle to filter for chlorophyll-a [WCHL]. The crews filter water for chlorophyll-*a* through a Whatman GF/F 47 mm 0.7 micron filter. Crews place the filter in a 50 mL tube, wrap the tube in a foil square and then place the tube in a zip-top plastic bag. The CHEM and WCHL samples are shipped on wet ice directly to the analysis laboratory in coolers.

6.2 Summary of Method

As an alternative to specifying laboratory methods for sample analysis, NWCA 2026 uses a performance-based approach that defines a set of laboratory method performance requirements for data quality. Method performance requirements for this project identify detection limit, precision, and accuracy objectives for each parameter. As described in **Section 6.6.7**, unless otherwise contractually bound by other requirements, the laboratory may choose to use any method that meets EPA's specifications for water chemistry measurements. If the lab chooses to use a different method other than the one outlined in this section, the lab will be required to send the SOP to the NWCA Laboratory Review Coordinator for review/approval as denoted in **Section 3.0**.

6.3 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions. In addition to the laboratory's usual requirements such as a Chemical Hygiene Plan, the laboratory must adhere to the following health and safety procedures:

- Laboratory facilities must properly store and dispose of solutions of weak acid.
- Laboratory personnel must wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves).
- When working with potential hazardous chemicals (e.g., weak acid), laboratory personnel must avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin and mucous membranes with acid. If skin contact occurs, remove clothing immediately. Wash and rinse the affected skin areas thoroughly with large amounts of water.

6.4 Definitions and Required Resources (Personnel, Laboratories, and Equipment)

This section provides definitions and required resources for using the procedure.

6.4.1 Definitions

The procedure uses the following terms:

Cl: Chloride

Detection Limit is the minimum concentration at which the analyte can be *detected* with confidence. In other words, the outcome can be reported with confidence that it is greater than zero (i.e., present in the sample) Also see "Sample-Specific Detection Limit" below.

DOC: Dissolved Organic Carbon

Duplicates are defined as two aliquots of the same sample which are analyzed separately using identical procedures. The results are used to evaluate the precision of the laboratory analyses.

FIA: Flow Injection Analysis

IC: Ion Chromatography

NH_3: Ammonia

NO_3-NO_2: Nitrate-nitrite

Percent Recovery: Recovery is measured by comparing the concentrations of a sample split into two parts; and one part is spiked with a known concentration value. C_s is the concentration measured in the spiked part; C is the concentration measured in the unspiked part; and s is the known concentration amount for the spike. The following equation is used to calculate the percent recovery:

$$\%Rs = \frac{C_s - C}{s} \times 100$$

Relative Standard Deviation (RSD): The precision at each concentration is reported in terms of the RSD. To calculate the RSD, first calculate the standard deviation, *S*, as follows:

$$S = \left[\frac{1}{n-1}\sum_{k=1}^{n}(C_s - \bar{C})^2\right]^{1/2}$$

where *n* is the number of replicate samples, *C*, is the concentration measure for the k[th] sample, and $\bar{C}$ is the average concentration of the replicate samples. Then, RSD is calculated as:

$$RSD = \left|\frac{S}{\bar{C}}\right| \times 100$$

Reporting Limit: A reporting limit is the point at which the measured value of the analyte can be reported with confidence.

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method's detection limit. For diluted samples, the sample-specific detection limit will be the product of the method's detection limit and the dilution factor.

Spiked Sample: See above, the Percent Recovery definition for purpose of spiked samples.

SO_4: Sulfate

TN: Total nitrogen

TP: Total phosphorus

6.4.2 General Requirements for Laboratories

Expertise. To demonstrate its competency/expertise to address each of the applicable parameters, the laboratory shall provide EPA with performance data demonstrating their proficiencies in analyzing water quality samples. See **APPENDIX D: LABORATORY REMOTE EVALUATION FORMS** for more information.

QA/QC requirements. To demonstrate its expertise in QA/QC procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include QMPs, QAPPs, and applicable SOPs. See **APPENDIX D: LABORATORY REMOTE EVALUATION FORMS** for more information.

To demonstrate its ongoing commitment to adhere to the requirements in the NWCA 2026 QAPP and LOM, the person(s) in charge of quality issues for the organization shall sign the NWCA QAPP Certification Page.

6.4.3 Equipment/Materials

The analytical method, selected by the laboratory, identifies the necessary equipment.

6.5 Sample Receipt

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery. When samples are received, if they are not logged in and processed immediately, they must be stored at 4 ^{o}C and processed within 48 hours. For each sampled site, the lab will receive the following samples on wet ice:

- One 1 liter bulk water sample labeled 'CHEM' for water chemistry analysis
- A filter in a 50 ml tube for chlorophyll-*a* labeled 'WCHL'

The laboratory technician must inspect the samples promptly on receipt and enter information onto a spreadsheet provided by the NARS IM Coordinator to log the samples into the NARS IM system within 24 clock hours. Steps in this process include:

1. Checking that each shipping container has arrived undamaged. Checking the temperature of one of the samples in the cooler using a thermometer that reads to at least -20 ºC (i.e., the expected temperature of frozen samples), or an infra-red (IR) temperature "gun" and recording the reading. Temperature of the wet ice shipments should be 4 ºC or less. Recording the condition and temperature of the sample on the sample tracking spreadsheet using the codes in **Table 6-2**.
2. Verifying that all required data elements, per **Table 6-2**, have been recorded in the tracking spreadsheet. If any data elements are missing, then enter them into the spreadsheet before submitting to NARS IM.
3. Transfering the samples for storage as follows:
    a. Water chemistry aliquots are prepared following the requirements in **Section 6.5** and then are stored in a refrigerator at 4° C in darkness.
    b. Chlorophyll-*a* filters to the freezer for no more than 28 days before analysis. Except during processing and analysis stages, the filter must be stored frozen to less than or equal -20 °C ± 2°.

Additionally, the laboratory technician must notify the EPA immediately about any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.

Table 6-2. Water chemistry login: required data elements.

Variable	Type	Description
SITE_ID	Character	Site identification code
SAMPLE_ID	Character	Sample number
DATE_COL	Date	Date that the field crew collected the sample
ANALYSIS_TYPE	Character	Water Chemistry (CHEM) or Chlorophyll-*a* (WCHL)
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory (will be on wet ice);
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control

Variable	Type	Description	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		NF	Sample is not at proper temperature
		Q	Other quality concerns, not identified above
CONDITION_COMMENT	Character	Explanation for Q FLAG (if needed)	

6.6 Preparation of Water Chemistry Aliquots

Figure 6-1 presents the sample preparation processing steps for the water chemistry indicators, including filtering and acidifying.

For nitrate-nitrite, DOC, ammonia, sulfate, and chloride, the laboratory technician will filter the sample before processing. The laboratory technician will conduct the following steps:

1. Use 0.4µm pore size polycarbonate filters for all filtrations.
2. Rinse vacuum filter funnel units thoroughly with ASTM Type I reagent water) five times before each use and in between samples. After placing a filter in the funnel unit, run approximately 100 mL of reagent water through the filter, with vacuum pressure, to rinse the filter. Discard the rinse water.
3. Place the appropriate sample bottle under the funnel unit and filter sample directly into the bottle. If a new filter is needed, remove the sample bottle, and rinse the new filter with 100 mL of reagent water before continuing.
4. Split the sample into two aliquots as shown in **Figure 6-1**.
5. Add ultra-pure acid (H_2SO_4, see **Table 6-3**) to one of the two aliquots. Cap the bottle tightly and invert the bottle several times to mix.
6. Store all aliquots in a refrigerator at 4°C in darkness.

For the other water chemistry analytes (TP, TN, turbidity, conductivity, and pH), the laboratory technician will complete the following steps:

1. Add ultra-pure acid (H_2SO_4,) to one unfiltered sample as show in **Figure 6-1** for TP and TN; and prepare one bottle without acid. Cap the TN-TP bottle tightly and invert the bottle several times to mix.
2. Store all aliquots in a refrigerator at 4°C in darkness.

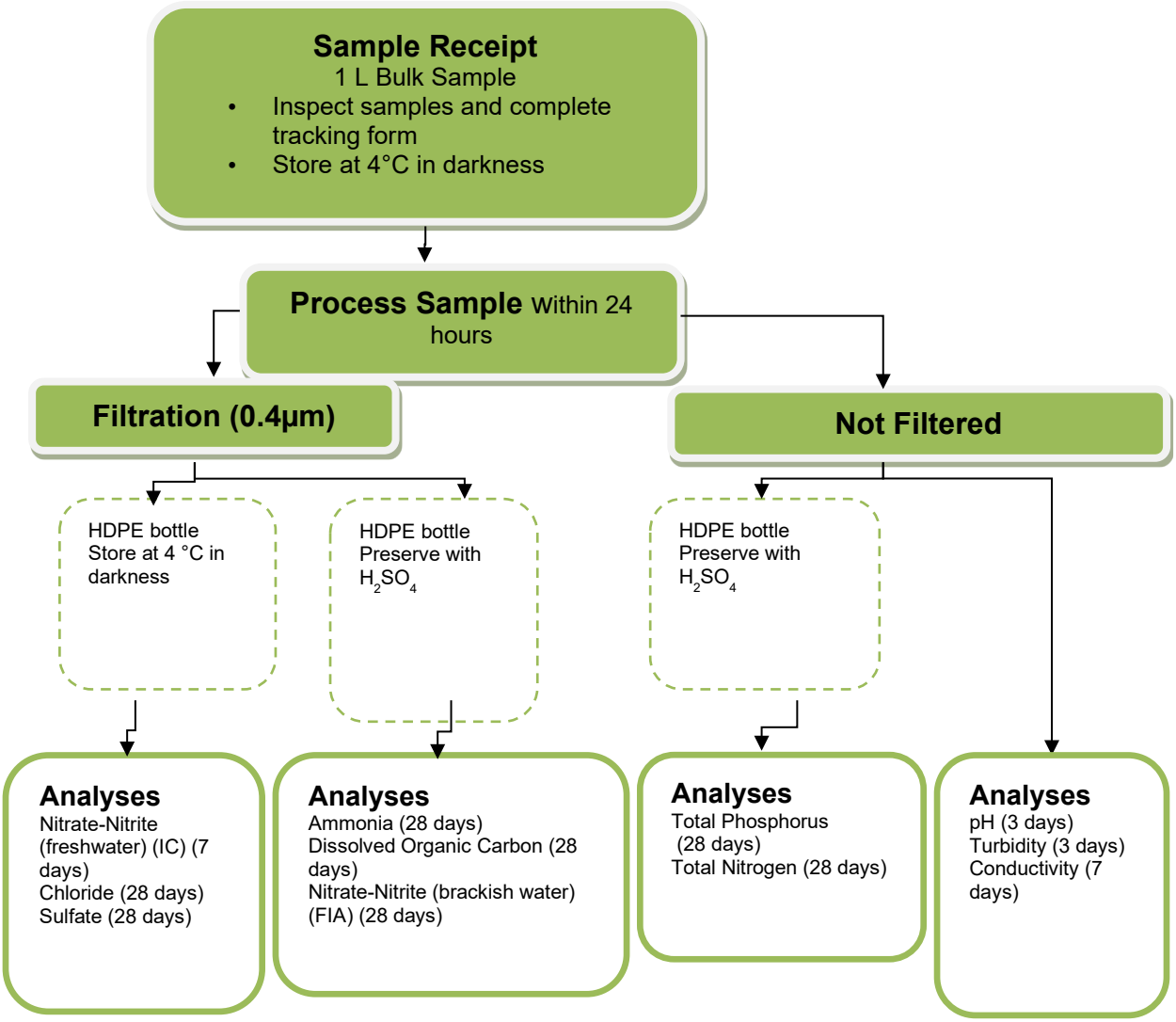


Figure 6-1. Water chemistry sample processing procedures.

Table 6-3. Water chemistry: acid preservatives added for various indicators.

H_2SO_4 used for the following indicators
DOC
NH_3
TN
TP
NO_3-NO_2 (when FIA method used)

6.7 Water Chemistry and Chlorophyll-*a* Analysis: Requirements

The laboratory shall perform analysis of the samples to determine the ammonia (NH_3), nitrate-nitrite (NO_3-NO_2), total nitrogen (TN), total phosphorus (TP), dissolved organic carbon (DOC), sulfate (freshwater samples only), chloride (freshwater samples only), conductivity, turbidity, pH, and chlorophyll-*a*. As an alternative to specifying laboratory methods for sample analysis (unless otherwise required by contract), NWCA uses a performance-based approach that defines a set of laboratory method performance requirements for data quality as shown in **Table 6-4**. Method performance requirements for this project identify the reporting limit, precision, and accuracy objectives for each parameter. NWCA is designating the reporting limit as the lowest value that the laboratory needs to quantify (as opposed to just detecting the parameter in the sample) and is equal to or below the value of the lowest non-zero calibration standard. EPA has set the value to double the long-term method detection limit (LT-MDL), following guidance presented in USGS (1999)[3].

NWCA expresses precision and accuracy objectives in both absolute and relative terms following Hunt and Wilson (1986). The transition value is the value at which performance objectives for precision and accuracy switch from absolute (≤ transition value) to relative (> transition value). For pH, the objectives are established for samples with higher and lower pH levels.

Table 6-4. Water chemistry and chlorophyll-*a*: laboratory method performance requirements.

Parameter	Units	Potential Range of Samples[4]	Method Detection Limit Objective[5]	Target Reporting Limit	Transition Value[6]	Precision Objective[7]	Accuracy Objective[8]
Conductivity	µS/cm at 25˚C	10 to 75,000	1.0	2.0	20	± 1 or ±10%	± 1 or 5%
pH	Std units	3 to 10	N/A	NA	5.75, 8.25	≤5.75 or ≥ 8.25 = ±0.07; 5.75-8.25 = ±0.15	≤5.75 or ≥ 8.25 =±0.05; 5.75-8.25 = ±0.10

[3] If a laboratory has questions related to meeting the LT-MDL, they may contact the NWCA Laboratory Review Coordinator to discuss concerns.

[4] Estimated from samples analyzed for NWCA 2011 and 2016.

[5] The method detection limit is determined as the minimum concentration of an analyte that can be measured and reported with 99% confidence (based on a one-sided 99% confidence interval) that the analyte is greater than zero.

[6] Value for which absolute (lower concentrations) vs. relative (higher concentrations) objectives for precision and accuracy are used.

[7] For duplicate samples, precision is estimated as the pooled standard deviation (calculated as the root-mean square) of all samples at the lower concentration range, and as the pooled percent relative standard deviation of all samples at the higher concentration range. For standard samples, precision is estimated as the standard deviation of repeated measurements across batches at the lower concentration range, and as percent relative standard deviation of repeated measurements across batches at the higher concentration range.

[8] Accuracy is estimated as the difference between the measured and target values of performance evaluation and/or internal reference samples at the lower concentration range, and as the percent difference at the higher concentration range.

Parameter	Units	Potential Range of Samples[4]	Method Detection Limit Objective[5]	Target Reporting Limit	Transition Value[6]	Precision Objective[7]	Accuracy Objective[8]
Ammonia (NH_3)	mg N/L	0 to 8	0.002	0.004	0.02	± 0.002 or ±10%	± 0.002 or ±10%
Nitrate-Nitrite (NO_3-NO_2)	mg N/L	0 to 25 (as nitrate)	0.002	0.004	0.02	± 0.002 or ±10%	± 0.002 or ±10%
Total Nitrogen (TN)	mg/L	0.04 to 70	0.01	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Phosphorus (TP)	µg P/L	0 to 20,000 (as TP)	2.0	4.0	20.0	± 2 or ±10%	± 2 or ±10%
Dissolved Organic Carbon (DOC)	mg C/L	0.5 to 200	0.1	0.20	1	± 0.10 or ±10%	± 0.10 or ±10%
Turbidity	Nephelometric Turbidity Units (NTU)	0.1 to 2000	1.0	2.0	10	± 1 or ±10%	± 1 or ±10%
Chloride (Cl)	mg Cl/L	0.06 to 40,000	0.03	0.06	0.3	± 0.03 or ±10%	± 0.03 or ±10%
Sulfate (SO_4)	mg SO_4/L	0.08 to 8,000	0.09	0.18	0.9	± 0.09 or ±10%	± 0.09 or ±10%
Chlorophyll-*a*	µg/L in extract	0.5 to 2,000	0.5	1.0	5	± 0.5 or ±10%	± 0.5 or ±10%

Table 6-5 summarizes analytical methods used for past NARS surveys for the selected parameters (EPA ORD-Corvallis). Participating laboratories may use alternative analytical methods for each target analyte as long as they can satisfactorily demonstrate the alternative method is able to achieve the performance requirements as listed in **Table 6-4**. **APPENDIX D: LABORATORY REMOTE EVALUATION FORMS** identifies the information that the laboratory should provide to the NWCA Laboratory Review Coordinator to use in determining whether the laboratories meet the necessary requirements.

Table 6-5. Water chemistry and chlorophyll-*a*: analytical methods used in past NARS surveys (EPA CORAL).

Analyte	Summary of Method[9]	References[10]	CORAL SOP[11]
pH (lab)	Automated, using ManSci PC-Titrate w/ Titra-Sip autotitrator and Ross combination pH electrode	EPA 150.1 (modified); SM4500H	16A.5
Conductivity	Electrolytic, Man-Tech TitraSip automated analysis OR manual analysis, electrolytic	EPA 120.1, SM2510	16A.5 11A.7
Nitrate+Nitrite, as N	Ion Chromatography (freshwater samples) OR FIA automated colorimetric (cadmium reduction for brackish or freshwater samples)	EPA 300.1; SW-846 9056A; SM4110B EPA 353.2 SM4500-NO_3-N-E Lachat 10-107-04-1-C	40A.9 36B.4
Chloride and Sulfate	Ion Chromatography (freshwater samples)	EPA 300.1; SW-846 9056A; SM4110B	40A.9
Ammonia, as N	FIA automated colorimetric (salicylate, dichloroisocyanurate) OR FIA automated colorimetric (phenolate method for brackish and seawater)	EPA 350.1 Lachat 10-107-06-3-D EPA 365.3 Lachat 31-107-06-1-B	30A.7 30B.3
Total nitrogen (TN)	Persulfate Digestion; FIA Automated Colorimetric Analysis (Cadmium Reduction, sulfanilamide)	EPA353.2 (modified) SM4500-N-C (modified) Lachat 10-107-04-1-C (modified)	34A.8
Total phosphorus (TP)	Persulfate Digestion	EPA 365.1	34A.8
Dissolved Organic Carbon (DOC)	UV promoted persulfate oxidation to CO_2 with infrared detection	SM5310C	21A.7
Turbidity	Nephelometric; Man-Tech TitraSip automated analysis, OR Manual analysis using Hach turbidimeter (high turbidity samples)	EPA 180.1, SM2130	16A.5 13A.6
Chlorophyll-*a* (WCHL)	Extraction 90% acetone analysis by fluorometry	EPA 445.0, EPA 446.0	71A.8

6.8 Data Entry

Table 6-6 identifies the required data elements that laboratories must provide to EPA, preferably in EPA's data template, available separately from EPA. **Table 6-7** identifies reporting units and significant figures.

[9] FIA=Flow injection analysis

[10] SM = Standard Methods for the Examination of Water and Wastewater, prepared by American Public Health Association (APHA). ASTM=American Society of Testing and Materials.

[11] CORAL= Corvalis Analytical Laboratory. References are to laboratory SOP being used at central laboratory. Available upon request from the EPA HQ Laboratory Review Coordinator.

Table 6-6. Water chemistry and chlorophyll-*a*: data elements for each sample.

Variable	Type	Description	
SITE_ID	Character	Site identification code or type of QC sample (e.g., LAB BLANK)	
SAMPLE_ID	Character	Sample number, LCS, QCCS, Blank, Matrix Spike, or CRM	
ANALYSIS_TYPE	Character	Water Chemistry (CHEM) or Chlorophyll-*a* (WCHL)	
REPEAT	Numeric	Duplicate	
DATE_COL	Date	Date that the field crew collected the sample	
DATE_RECEIVED	Text	Date sample was received by lab	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		NF	Sample is not at proper temperature
		Q	Other quality concerns, not identified above
CONDITION_COMMENT	Character	Explanation for Q FLAG (if needed)	
PARAMETER	Character	Analyte name	
CAS_NO	Character	CAS Registry number	
LABNAME	Character	Laboratory name (abbreviation)	
METHOD	Character	Laboratory method used	
ANALYST	Character	Last name or initials of person who performed the analysis	
REVIEWER	Character	Last name or initials of the person who provided a separate independent review of the data	
INSTRUMENT	Character	Identification of instrument used for the analysis – provide enough information to identify the particular instrument in the laboratory	
DATE_PROCESSED	Date	Date that the analysis started	
QC_BATCH_LOT	Character	Unique laboratory QC lot numbers must be assigned to each batch of samples. The lot number must associate each batch of field samples to the appropriate laboratory control sample, matrix spike, laboratory duplicate, method blank, and CRM samples.	
HOLDING_TIME	Y/N	Analysis performed within holding time	
MATRIX	Character	Water	
MDL	Numeric	Lab method detection limit (based upon lab's historical data)	
LRL	Numeric	Lab reporting limit (based upon lab's historical data)	
DILUTION	Numeric	Dilution of sample (blank or 1 if no dilution)	
RESULT	Numeric	Concentration value	
RESULT_QUAL	Character	Data qualifier (usually blank)	
RESULT_REASON	Character	Reason for qualification in RESULT_QUAL (usually blank)	
UNIT	Character	Unit of measurement for RESULT, MDL, and LRL	
QC_CODE	Character	Apply laboratory defined QC codes and describe in the comments field. Provide set of laboratory's code as part of the case narrative	
QC_COMMENT	Character	Explain situation that created QC code, or any unusual aspects of the analysis	

Table 6-7. Water chemistry: reporting units and significant figures.

Measurement	Units	Minimum No. Significant Figures
Temperature	°C	2
pH	pH units	3
Dissolved Organic Carbon	mg/L	3
Conductivity	µS/cm at 25 °C	3
Total phosphorus	µg P/L	3
Total nitrogen	mg N/L	3
Nitrate-Nitrite	mg N/L	3
Ammonia	mg N/L	3
Turbidity	NTU	3
Chlorophyll-*a*	ug/L	3
Chloride	mg/L	3
Sulfate	mg/L	3

6.9 Quality Measures

This section describes the QA/QC measures used to ensure that the data will meet NWCA's requirements. QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. The laboratory must conduct QC analyses for each batch of samples. Each batch shall consist of no more than 20 samples. Unique laboratory QC lot numbers must be assigned to each batch of samples. The lot number must associate each batch of field samples to the appropriate measures such as laboratory control sample, matrix spike, laboratory duplicate, and method blank samples. Also, each laboratory QC samples (i.e., preparation and instrument blanks, laboratory control sample (LCS), spike/duplicate, etc.) must be given a unique sample identification. **Table 6-8** provides a summary of the QC requirements.

Table 6-8. Water chemistry and chlorophyll-a: QC activities for water quality samples.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Demonstrate competency for analyzing water samples to meet the performance measures	All	Demonstration of past experience with water samples in achieving the method detection limits	Once	See APPENDIX D: LABORATORY REMOTE EVALUATION FORMS	EPA will not approve any laboratory for NWCA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can demonstrate competency for its NWCA samples.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Check condition of sample when it arrives.	All	Sample issues such as cracked container(s); missing label(s); temperature; adherence to holding time requirements; sufficient volume for test.	Once	No sample issues or determination that sample can still be analyzed	Lab determines if the sample can be analyzed or has been too severely compromised (e.g., contamination). Assign appropriate condition code identified in **Table 6-6**..
Store sample appropriately.	All	Check the temperature of the refrigerator per laboratory's SOPs.	Record temperature of samples upon arrival at the laboratory. Check temperature of the refrigerator/freezer where samples are stored at least daily if using a continuous temperature logger and twice daily (once at beginning of the day and once at the end) not using a continuous logger.	While stored at the laboratory, the sample must be kept at 0-6° C (for aliquots except chlorophyll-a) and -20° C for the chlorophyll-a sample.	If at any time samples are warmer than required, note temperature and duration (either from the continuous temperature log or from the last manual reading) in comment field identified in **Table 6-6**. Lab will still perform test. EPA expects that the laboratory will exercise every effort to maintain samples at the correct temperature.
Analyze sample within holding time	All			The test must be completed within the holding time specified in the analytical method.	Perform test in all cases but note reason for performing test outside holding time. Add QC Code for any samples analyzed outside of holding time in **Table 6-6.** EPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Laboratory/ Reagent Blank	All	ASTM Type I reagent water For dissolved analytes: Process through filtration unit	Once per week	All analytes < RL (**Table 6-4**)	Prepare and analyze new blank. Determine and correct problem (e.g., reagent contamination, instrument calibration, or contamination introduced during filtration) before proceeding with any sample analyses. Reestablish statistical control by analyzing three blank samples.
Filtration Blank	All dissolved analytes	ASTM Type I reagent water processed through filtration unit	Prepare filter blank for each lot of filters and examine the results before any other filters are used from that box	All analytes < RL (T**able 6-4**)	Measure archived samples if review of other laboratory blank information suggest source of contamination is sample processing.
LT-MDL check standard	All analytes requiring MDL studies		Once per analytical batch	Within accuracy objective (**Table 6-4**)	Confirm achieved LRL by repeated analysis of LT-MDL check standard. Evaluate affected samples for possible re-analysis.
Initial and Continuing Calibration Verification (ICV and CCV)	All		Analyze ICV after calibration. Analyze CCV after every 10 samples and at end of analytical batch	±10% or method criteria	Perform corrective action and reanalyze all associated samples since last successful CCV. Alternatively, recalibrate and reanalyze all samples since last successful CCV.
Initial and Continuing Calibration Blank (ICB and CCB)	All	Reagent Water	Analyze ICB immediately after the ICV. Analyze CCB immediately after every CCV	All analytes < RL	Perform corrective action and reanalyze all associated samples since the last successful CCB. Alternatively, recalibrate and reanalyze all samples since last successful CCB.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Analytical Duplicate Sample	All		One per 10 samples	Within precision objective (**Table 6-4**)	If results are below LRL: Prepare and analyze duplicate from different sample (volume permitting). Review precision of batch. Check preparation of duplicate sample. Qualify all samples in batch for possible reanalysis.
Standard Reference Material (SRM)	When available for a particular indicator		One analysis in a minimum of five separate batches	Manufacturers certified range	Analyze standard in next batch to confirm suspected inaccuracy. Evaluate calibration and QCCS solutions and standards for contamination and preparation error. Correct before any further analyses of routine samples are conducted. Reestablish control by three successive reference standard measurements that are acceptable. Qualify all sample batches analyzed since the last acceptable reference standard measurement for possible reanalysis.
Matrix Spike Samples	Only prepared when samples with potential for matrix interferences are encountered		One per batch	80-120% Recovery	Select two additional samples and prepare fortified subsamples. Reanalyze all suspected samples in batch by the method of standard additions. Prepare three subsamples (unfortified, fortified with solution approximately equal to the endogenous concentration, and fortified with solution approximately twice the endogenous concentration).

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Use consistent units for QC samples and field samples	All	Verify that all units are provided consistently within each indicator.	Data reporting	For each indicator, all field and QC samples are reported with the same measurement units	If it is not possible to provide the results in consistent units, then assign a QC code and describe the reason for different units in the comments field of the database.
Maintain completeness	All	Determine completeness	Data reporting	Completeness objective is 95% for all indicators (useable with or without flags).	Contact EPA HQ NWCA Laboratory Review Coordinator* immediately if issues affect laboratory's ability to meet completeness objective.

***Section 2.0** provides contact information for the EPA HQ NWCA Laboratory Review Coordinator. Laboratories under contract to EPA must contact the TOCOR instead of the Laboratory Review Coordinator.

6.10 Sample and Record Retention

The laboratory shall retain:

- The sample materials for a minimum of 1 year after collection. During this time, the laboratory shall store the materials cold (e.g., 4 ° C) and in darkness. The lab shall retain the sample materials from the 1-year point until the EPA publishes the final report at ambient temperatures.
- Original records, including laboratory notebooks for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

6.11 References

Hunt, D.T.E. and A.L. Wilson. 1986. The Chemical Analysis of Water: General Principles and Techniques. 2nd ed. Royal Society of Chemistry, London, England.

USEPA. 1997. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices – 2nd Edition. EPA No. 600-R-97-072. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA. September 1997. Method 353.4 "Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis, Revision 2.0.

USGS. 1999. "New reporting procedures based on long-term method detection levels and some considerations for interpretations of water-quality data provided by the U.S. Geological Survey National Water Quality Laboratory." Open-File Report: 99-193 by Childress, Oblinger, et al..

Youden, W.J. 1969. Ranking laboratories by round-robin tests. In Precision Measurement and Calibration. H.H. Ku, ed. NBS Special Publication 300, Vol. 1. U.S. GPO Washington, D.C.

USEPA. 2011. National Wetland Condition Assessment. Laboratory Operations Manual. EPA843-R10-002. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2011. National Wetland Condition Assessment. Quality Assurance Project Plan. EPA843-R10-003. U.S. Environmental Protection Agency, Washington, DC.

7.0 MICROCYSTINS IMMUNOASSAY PROCEDURE

7.1 Introduction

This section describes an immunoassay procedure that measures concentrations of total microcystins in water samples using Gold Standard Diagnostics Microcystins-ADDA Test Kits ("kits")[12]. Each kit is an enzyme-linked immunosorbent assay (ELISA) for the determination.

Microcystins refers to the entire group of toxins, all of the different congeners, rather than just one congener. Cyanobacteria can produce one or many different congeners at any one time, including Microcystin-LR (used in the kit's calibration standards), Microcystin-LA, and Microcystin-RR. The different letters on the end signify the chemical structure (each one is slightly different), which makes each congener different.

Field Collection/Sample Summary: Crews collect a microcystins sample in a 125-mL bottle. Frozen microcystins samples will be shipped on dry ice from the field crews to the contract batching laboratory (unless the sample is being processed by a state or other non-NWCA national laboratory). The contract batching laboratory will send the batched frozen samples to the analysis laboratory in coolers on ice where they can be held in a freezer until ready for analysis (keeping in mind the 90-day holding time). If a state or other non-NWCA national laboratory is processing the samples, crews may ship or hand deliver the frozen samples to their lab based on internal procedures.

7.2 Summary of Method

The procedure is an adaption of the instructions provided by Gold Standard Diagnostics for determining total microcystins concentrations using its ELISA-ADDA kits. For freshwater samples, the procedure's reporting range is 0.15 µg/L to 5.0 µg/L, although, theoretically, the procedure can detect, not quantify, microcystins concentrations as low as 0.10 µg/L. For samples with higher concentrations of microcystins, the procedure includes the necessary dilution steps. The procedure also provides additional sample preparation steps for samples with salinities ≥3.5 parts per thousand (ppt). The results then are adjusted by a factor of 1.75 for a reporting range of 0.263 µg/L to 8.75 µg/L.

7.3 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions, because the kit substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. In addition to the laboratory's usual requirements such as a Chemical Hygiene Plan, the laboratory must adhere to the following health and safety procedures:

- Laboratory facilities must properly store and dispose of solutions of weak acid.
- Laboratory personnel must wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves).

[12] Gold Standard Diagnostics, "Microcystins-ADDA ELISA (Microtiter Plate): Product No.520011" Retrieved on November 5, 2020 from https://www.goldstandarddiagnostics.com/pub/media/productattachments/files/m/i/microcystins_nodularins-adda-elisa-user-guide-520011.pdf

- When working with potential hazardous chemicals (e.g., weak acid), laboratory personnel must avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin and mucous membranes with the TMB and stopping solution. If skin contact occurs, remove clothing immediately. Wash and rinse the affected skin areas thoroughly with large amounts of water.

7.4 Definitions and Required Resources (Laboratories and Equipment)

This section provides definitions and required resources for using the procedure.

7.4.1 Definitions

The following terms are used throughout the procedure:

Absorbance (A) is a measure of the amount of light in a sample. A standard statistical curve is used to convert the absorbance value to the concentration value of microcystins.

Brackish and Seawater Samples, for the purposes of the Gold Standard Diagnostics (formerly Abraxis) microcystins test procedure, are samples with salinity greater than or equal to 3.5 ppt. (EPA is using different definitions for the water chemistry samples.) EPA recognizes that brackish water is usually defined as 0.5 ppt, and seawater as 35 ppt, but for this immunoassay procedure, it is important to use additional steps described in **Section 7.6.2** for any sample with salinity greater than or equal to 3.5 ppt. The sample labels provide the salinity levels.

Calibration Range is the assay range for which analysis results can be reported with confidence. For undiluted samples, it ranges from the reporting limit of 0.15 µg/L to a maximum value of 5.0 µg/L. Values outside the range are handled as follows.

Given the salinity is less than 3.5 ppt, if the result value is:

- Less than 0.10 µg/L, then the laboratory reports the result as is and flags the sample as being a non-detect (DATA_FLAG=ND).
- Between 0.10 µg/L and the reporting limit of 0.15 µg/L (i.e., >=0.10 µg/L and <0.15 µg/L), the laboratory should record the value, but assign a QC code to the value (i.e., DATA_FLAG=J).
- 5.0 µg/L or greater, the laboratory must flag the samples as HI, leave the CONC column blank, dilute and reanalyze the sample (DATA_FLAG= HI).

Given the salinity greater than or equal to 3.5 ppt, the technician must follow the seawater cleanup procedures in **Section 7.6.2** and then analyze the sample. The results are then adjusted by a factor of 1.75 for a reporting range of 0.263 µg/L to 8.75 µg/L. If the adjusted result value is:

- Less than 0.175 µg/L, then the laboratory reports the result as is and flag the sample as a non-detect (DATA_FLAG=ND).
- Between 0.175 µg/L and the reporting limit of 0.263 µg/L (i.e., >=0.175 and <0.263 µg/L), the laboratory should record the value but assign a QC code to the value (i.e., DATA_FLAG=J).
- Greater than 8.75 µg/L, the laboratory must flag the data as “HI” in the Data Flag, leave the CONC column blank, dilute and reanalyze the sample (DATA_FLAG= HI).

Coefficient of Variation (CV): The precision for a sample is reported in terms of the percent CV of its absorbance values. To calculate the %CV, first calculate *S* (standard deviation) as follows:

$$S = \left[\frac{1}{n-1}\sum_{i=1}^{n}(A_i - \bar{A})^2\right]^{1/2}$$

where *n* is the number of replicate samples, A_i, is the absorbance measured for the i^{th} replicate. Samples are evaluated in duplicate (i=1 or 2); controls are either evaluated in duplicate or triplicate (i=1, 2, 3). $\bar{A}$ is the average absorbance of the replicates. Then, calculate %CV as:

$$\%CV = \left|\frac{S}{\bar{A}}\right| \times 100$$

Dark or Dimly Lit: Away from sunlight, but under incandescent lighting is acceptable.

Duplicate samples (D) are defined as the second aliquots of an individual sample within a well plate. Each sample, including the standards, are run in pairs and both results for the primary (P) and duplicate aliquot are reported in the result column of the lab deliverable.

Method Detection Limit is the minimum concentration at which the analyte can be *detected* with confidence. In other words, the outcome can be reported with confidence that it is greater than zero (i.e., present in the sample). The detection limit is less than the reporting limit of 0.15 µg/L at which the *measured* value of the analyte can be reported with confidence. Also see "Sample-Specific Detection Limit."

Primary samples (P) are defined as the first aliquot of a sample within a well plate. Each sample is analyzed in pairs. The results of both the primary aliquot and secondary, duplicate aliquot are reported in the result column of the lab deliverable.

Relative Standard Deviation (RSD) is the same as the coefficient of variation (%CV). Because many of the plate reader software programs provides the CV in their outputs, the procedure presents the QC requirement in terms of %CV instead of RSD.

Reporting Limit: A reporting limit is the point at which the measured value of the analyte can be reported with confidence. For undiluted samples with a salinity <3.5 ppt, the reporting limit is 0.15 µg/L, for undiluted samples with a salinity ≥3.5 ppt, the reporting limit is 0.263 µg/L. The reporting limit for diluted samples is the product of the result multiplied by the dilution factor.

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method's detection limit (salinity <3.5 ppt = < 0.10 µg/L or salinity ≥ 3.5 ppt = <0.175 ug/L). For diluted samples, the sample-specific detection limit will be the product of the method's detection limit and the dilution factor. Typical values for the dilution factor will be 10 or 100.

Seawater Sample: See definition for brackish and seawater samples.

Standard Deviation (S) shows variation from the average

7.5 General Requirements for Laboratories

7.5.1 Expertise

To demonstrate its expertise, the laboratory shall provide EPA with one or more of the following:

- Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.
- Documentation detailing the expertise of the organization, including professional certifications for water-related analyses, membership in professional societies, and experience with analyses that are the same or similar to the requirements of this method.

7.5.2 QA/QC requirements

To demonstrate its expertise in QA/QC procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include QMPs, QAPPs, and applicable SOPs.

To demonstrate its ongoing commitment to adhere to the requirements in the NWCA 2026 QAPP and LOM, the person in charge of quality issues for the organization shall sign the NWCA 2026 QAPP Certification Page.

7.5.3 Equipment/Materials

The procedures require the following equipment and information:

- Gold Standard Diagnostics ADDA Test Kit, Product #520011
- Adhesive Sealing Film (Parafilm) for Micro Plates (such as Rainin, non-sterile, Cat. No. 96-SP-100): Used to cover plates during incubation
- Data Template – See **Figure 7-1** Microcystins: sample template
- Distilled or Deionized Water: For diluting samples when necessary
- ELISA evaluation software
- Glass scintillation, LC, vials (two vials of 20 mL each)
- Glass vials with Teflon-lined caps of size:
    - 20 mL
    - 4 mL (for dilutions)
- Multichannel Pipette & Tips: A single-channel and an 8-channel pipette are used for this method
- Norm-ject syringes (or equivalent)
- Paper Towels: For blotting the microtiter plates dry after washing
- Permanent Marker (Sharpie Fine Point): For labeling samples, bottles, plates and covers

- Plate Reader (e.g., Metertech Model M965 AccuReader; ChroMate®; or equivalent readers with software to read the microtiter plates and measure absorbances)
- Reagent Reservoirs (e.g., Costar Cat Number 4870): Plain plastic reservoir for reagents that accommodate the use of a multi-channel pipette.
- Test tubes: For dilutions, if needed
- Timer: For measuring incubation times
- Vortex Genie: For mixing dilutions
- Whatman Glass fiber syringe filter (25mm, GF 0.45 µm filter)

Analysis of samples with salinity ≥3.5 ppt require additional equipment and supplies, as follows:

- Microcystins-ADDA Seawater Sample Clean-Up Kit (Product #529912) which includes the following supplies:
    - Disposable 5 ¾" glass Pasteur pipettes
    - Disposable 9" glass Pasteur pipettes
    - Glass wool
    - Pasteur pipette bulb
    - Microcystins-ADDA Seawater Sample Treatment Solution
    - Microcystins-ADDA Seawater Sample Clean-up Resin
- 12x75 mm test tubes
- Scoopula
- Micropipettes with disposable plastic tips
- Vortex mixer

7.6 Sample Receipt

Field crews keep the microcystins samples cool while in the field and then pack the samples in ice for delivery to a central facility ("batching laboratory") or the State's laboratory. The batching and state laboratories freeze the samples upon receipt. Periodically, the batching laboratory ships samples to the microcystins laboratory. The batching and microcystins laboratory may retain the frozen samples for several months (up to 90 days from collection) before analysis.

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery.

The laboratory technician must inspect the samples promptly on receipt and enter information onto a spreadsheet provided by the NARS IM Coordinator to log the samples into the NARS IM system within 24 clock hours. Steps in this process include:

1. Inspecting each sample THE SAME DAY THEY ARE RECEIVED:
    a. Verifying that the sample IDs in the shipment match those recorded on the:
        i. Chain of custody forms when the batching laboratory sends the samples to the microcystins laboratory; or

ii. Sample tracking form if the field crew sends the shipment directly to the State laboratory.

b. Recording the information in **Table 7-1** into the NARS IM sample tracking spreadsheet, including the Condition Code for each sample.

2. Storing samples in the freezer until sample preparation begins.
3. Maintaining the chain of custody or sample tracking forms with the samples.

Additionally, the laboratory technician must notify the EPA immediately about any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.

Table 7-1. Microcystins indicator: required data elements – login.

FIELD	FORMAT	DESCRIPTION	
LAB ID	text	Name or abbreviation for QC laboratory	
DATE RECEIVED	MMDDYY	Date sample was received by lab	
SITE ID	text	NWCA site ID as used on sample label	
VISIT NUMBER	numeric	Sequential visits to site (1 or 2)	
SAMPLE ID	numeric	Sample id as used on field sheet (on sample label)	
DATE COLLECTED	MMDDYY	Date sample was collected	
CONDITION CODE	text	Condition codes describing the condition of the sample upon arrival at the laboratory.	
		Flag	**Definition**
		Blank or N	Not a sample (Blank, standard or control)
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		NF	Sample is not frozen
		Q	Other quality concerns, not identified above
CONDITION COMMENT	text	Comments about the condition of the sample. Required for “Q”. Optional for others.	

7.7 Procedure

The following sections describe the sample and kit preparation and analysis.

7.7.1 Sample Preparation

For each frozen sample (125 mL per sample), the laboratory technician runs it through a freeze-thaw cycle three times to lyse the cells as follows:

1. All cycles: Keep the samples in dark or dimly lit areas (i.e., away from sunlight, but under incandescent lighting is acceptable).
2. First freeze-thaw cycle:
   a. Start with a frozen 125 ml sample.

b. Thaw the sample to room temperature (approximately 25° C). Swirl the sample to check for ice crystals. At this temperature, no ice crystals should be present in the sample.
c. Shake well to homogenize the sample, then transfer 10 mL to an appropriately labeled clean 20 mL glass vial.

3. Second freeze-thaw cycle:

   a. Freeze the vial.
   b. Keep the large sample bottle (from the 125 mL initial sample) frozen for future use.
   c. Thaw the sample vial contents to room temperature.

4. Third freeze-thaw cycle:

   a. Freeze the vial.
   b. Thaw the vial contents to room temperature.
   c. Filter the vial contents through a new, syringe filter (0.45 µm) into a new, labeled 20 mL glass scintillation vial. Norm-ject syringes and Whatman Glass fiber syringe filters (25mm, GF 0.45 µm filter) or similar alternative are acceptable. One new syringe and filter should be used per sample.

7.7.2 Additional Sample Preparation for Samples with Salinity>3.5 ppt

For any sample with salinity of 3.5 ppt or greater, the laboratory technician needs to perform the following additional steps provided by Gold Standard Diagnostics Conductivity will be collected as a proxy for salinity during processing of the water chemistry samples. These values will be provided back to NWCA HQ Team on a weekly basis which will then be redistributed to the microcystins laboratories. Labs must convert this conductivity value into salinity prior to analyzing the samples. For all samples with salinity less than 3.5 ppt, the technician skips this section (i.e., **Section 7.7.2**) and goes directly to kit preparation as described in **Section 7.7.3**. For samples with salinity ≥3.5 ppt the technician:

1. Prepares the column as follows:

   a. Place a small amount of glass wool into the top of a 5 ¾” glass Pasteur pipette. Using a 9” glass Pasteur pipette, push the glass wool into to the bottom of the 5 ¾” pipette to form the base of the column. The depth of the glass wool should be approximately 5 mm. Place the column into a 12x75 mm test tube.

   b. Each column will require approximately 1.5 g of Seawater Sample Clean-Up Resin. Calculate and add the appropriate amount of Microcystins-ADDA Seawater Sample Clean-Up Resin to a 20 mL glass vial.

   c. Add distilled or deionized water at an approximately 2:1 ratio to the Microcystins- ADDA Seawater Sample Clean-Up Resin (for example, 10 mL of deionized or distilled water per 5 g of Resin). Shake or vortex.

   d. Pipette the Resin in water solution into the column using the 9” Pasteur pipette. Avoid the formation of air bubbles in the column bed by keeping the tip of the pipette at the surface of the bed being created. Fill the column to the indentation approximately 2 cm from the top of the pipette. This will create an approximately 8 cm column.

   e. Allow the deionized or distilled water to drain from the column. Lift the tip of the column at least 1 cm above the surface of the water in the tube. Place the pipette bulb against the top of the column (do not attach the bulb to the column) and push the remaining water out of

the column. Avoid allowing the tip of the column to come into contact with the water in the tube to prevent aspiration of water back into the column.

f. Place the column into an appropriately labeled 4 mL glass vial.

2. Cleans up the sample as follows:

a. Add 1 mL of the sample to a clean, appropriately labeled 4 mL glass vial. Add 50 µL of Microcystins-ADDA Seawater Sample Treatment Solution. Vortex.

b. Add 375 µL of the treated sample to the top of the column. Allow the sample to drain through the column and collect in the vial.

c. Add a second 375 µL aliquot of the treated sample to the column. Allow to drain through the column.

d. Lift the tip of the column at least 1 cm above the surface of the sample in the vial. Place the pipette bulb against the top of the column (do not attach the bulb to the column) and push the remaining sample out of the column. Avoid allowing the tip of the column to come into contact with the sample in the vial to prevent aspiration of the sample back into the column.

e. Lower the column back into the vial. Add 500 µL of distilled or deionized water to the top of the column. Allow the rinse to drain through the column and collect with the sample.

f. Lift the tip of the column at least 1 cm above the surface of the sample/rinse in the vial. Place the pipette bulb against the top of the column (do not attach the bulb to the column) and push the remaining rinse out of the column. Avoid allowing the tip of the column to come into contact with the sample in the vial to prevent aspiration of the sample back into the column.

g. Remove the column and discard (columns are single use only). Cap vial and vortex. The sample can then be analyzed using the Eurofins Technologies (formerly Abraxis) Microcystins-ADDA ELISA Kit beginning with **Section 7.7.3**.

7.7.3 Kit Preparation

The technician prepares the kits using the following instructions:

1. Check the expiration date on the kit box and verify that it has not expired. If the kit has expired, discard and select a kit that is still within its marked shelf life. (Instead of discarding the kit, consider keeping it for training activities.)
2. Verify that each kit contains all the required contents:

a. Microtiter plate

b. Standards (6) referenced in this procedure as follows with the associated concentration:

i. S0: 0 µg/L
ii. S1: 0.15 µg/L
iii. S2: 0.40 µg/L,
iv. S3: 1.0 µg/L
v. S4: 2.0 µg/L
vi. S5: 5.0 µg/L

c. Kit Control (KC): 0.75 µg/L

d. Antibody solution

e. Anti-Sheep-HRP Conjugate

f. Wash Solution 5X Concentrate

g. Color Solution

h. Stop Solution

i. Diluent

j. Foil bag with 12 microtiter plate strips

3. If any bottles are missing or damaged, discard the kit. This step is important because Gold Standard Diagnostics has calibrated the standards and reagents separately for each kit.
4. Adjust the microtiter plate, samples, standards, and the reagents to room temperature.
5. Remove 12 microtiter plate strips (each for 8 wells) from the foil bag for each kit. The plates contain 12 strips of 8 wells. If running less than a whole plate, remove unneeded strips from the strip holder and store in the foil bag, ziplocked closed, and place in the refrigerator.
6. Store the remaining strips in the refrigerator (4-8^{o} C).
7. Prepare a negative control (NC) using distilled water
8. The standards, controls, antibody solution, enzyme conjugate, color solution, and stop solutions are ready to use and do not require any further dilutions.
9. Dilute the wash solution with deionized water. (The wash solution is a 5X concentrated solution.) In a 1L container, dilute the 5X solution 1:5 (i.e., 100 mL of the 5X wash solution plus 400 mL of deionized water). Mix thoroughly. Set aside the diluted solution to wash the microtiter wells later.
10. Handle the stop solution containing diluted H_2SO_4 with care.

7.7.4 Insertion of Contents into Wells

This section describes the steps for placing the different solutions into the 96 wells. Because of the potential for cross contamination using a shaker table, the following steps specify manual shaking of the kits instead of mechanized shaking.

1. While preparing the samples and kit, turn the plate reader on so it can warm up. The plate reader needs a minimum of 30 minutes to warm up.
2. Turn on the computer so that it can control and access the plate reader.
3. Print the template (**Figure 7-1**) to use as reference when loading the standards, controls, and samples as described in the next step. Templates contain rows, labeled with a marking pen, of strips of 8 wells that snap into the blank frame. (If the laboratory wishes to use a different template, provide a copy to the EPA HQ Laboratory Review Coordinator for approval prior to first use. (See **Table 2-1** for contact information.)
4. Using the 100-µL pipette, add 50 µL, each, of the standards, controls, and samples to the appropriate wells in the plate. Place all six standards (0.00, 0.15, 0.40, 1.00, 2.0 and 5.0 µg/L), the kit control (0.75 µL), and negative control, in pairs, starting in the well in the upper left-hand

corner of the kit as shown in **Figure 7-1**. Verify that the software displays the same template or make any necessary corrections.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S4	P1	P5	P9	P13	P17	P21	P25	P29	P33	P37
B	S0	S4	D1	D5	D9	D13	D17	D21	D25	D29	D33	D37
C	S1	S5	P2	P6	P10	P14	P18	P22	P26	P30	P34	P38
D	S1	S5	D2	D6	D10	D14	D18	D22	D26	D30	D34	D38
E	S2	KC	P3	P7	P11	P15	P19	P23	P27	P31	P35	P39
F	S2	KC	D3	D7	D11	D15	D19	D23	D27	D31	D35	D39
G	S3	NC	P4	P8	P12	P16	P20	P24	P28	P32	P36	P40
H	S3	NC	D4	D8	D12	D16	D20	D24	D28	D32	D36	D40

Figure 7-1. Microcystins indicator: sample template.

Key: S0-S5 = Standards; KC = Control supplied with Kit (i.e., Kit Control);
NC = Negative Control (Laboratory Reagent Blank);
P = Primary aliquot for each unknown sample collected by field crew;
D= “DUPLICATE” aliquot for each matching unknown Primary sample.

5. Add 50 µL of the pink antibody solution to each well using the multi-channel pipettor and a reagent reservoir. Use dedicated reagent reservoirs for each reagent to avoid contamination from one reagent to another.
6. Place the sealing Parafilm over the wells.
7. Manually mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
8. Place the plate in an area away from light for 90 minutes.
9. After 90 minutes, carefully remove the Parafilm.
10. Empty the contents of the plate into the sink, pat inverted plate dry on a stack of paper towels, and then wash the wells of the plate three times with 250 µL of washing solution using the multi-channel pipette. After adding the washing solution each time, empty the solution into the sink and use the paper towels as before.
11. Add 100 µL of enzyme conjugate solution to all wells using the multi-channel pipettor.
12. Cover the wells with Parafilm.
13. Manually mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
14. Place the strip holder in an area away from light for 30 minutes.
15. After 30 minutes, remove the Parafilm, decant, and rinse the wells three times again with 250 µL of washing solution as described in step 10.
16. Add 100 µL of color solution to the wells using the multi-channel pipette and reagent reservoir. This color solution will make the contents have a blue hue.
17. Cover the wells with Parafilm.

18. Manually mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
19. Place the plate in an area away from light for 20 minutes.
20. After 20 minutes, remove the Parafilm and add 50 µL of stopping solution to the wells in the same sequence as for the color solution. This will turn the contents a bright yellow color. After adding the stopping solution, read the plate within 15 minutes.
21. Within 15 minutes of adding the stopping solution, use the microplate ELISA photometer (plate reader) to determine the absorbance at 450 nm. The software (i.e., commercial ELISA evaluation program) calculates the absorbance and concentration values of the samples from the calibration curve and the average values for each pair. Use a 4-parameter standard curve fit to determine the concentrations.
22. Dispose of solution in plates in a lab sink. Rinse plates and sink with water to dilute the weak acid present.
23. Perform QC evaluations of the data as follows:
    a. If the following failures occur, then the laboratory must reanalyze all samples in the analytical run:
        i. Standard curve with a correlation coefficient of less than 0.99 (i.e., R<0.99)
        ii. Standards S0-S5 must have decreasing absorbance values. First, calculate the average values for each standard. That is, if $\bar{A}_i$ is the absorbance average for S_i, then the absorbance averages must be:
        $\bar{A}_0 > \bar{A}_1 > \bar{A}_2 > \bar{A}_3 > \bar{A}_4 > \bar{A}_5$
        iii. The average absorbance of the standard S0 less than 0.8 (i.e., $\bar{A}_0 < 0.8$).
        iv. Two or more negative control samples with detectable concentrations of microcystins (i.e., values > 0.1 µg/L). If this occurs, then evaluate possible causes (e.g., cross-contamination between samples), and if appropriate, modify laboratory processes before the next analytical run.
        v. Results for control samples of outside the acceptable range of 0.75 +/- 0.185 ppb. That is, results must be between 0.565 and 0.935.
    b. If either, or both, of the following failures occur, then the sample must be reanalyzed (maximum of two analyses, consisting of the original analysis and, if necessary, one reanalysis):
        i. The concentration value registers as HIGH (exceeds the calibration range). Dilute the sample for the reanalysis per **Section 7.7.6**.
        ii. The %CV > 15% between the duplicate absorbance values for a sample.
24. If the sample has a salinity of 3.5 ppt or greater, then convert the results by multiplying by 1.75. If the assay was non-detected, then the sample-specific detection limit is 0.175 µg/L. The reporting limit is 0.263 µg/L. The calibration range is 0.263 µg/L to 8.75 µg/L.
25. Record the results, even if the data failed the QC requirements in #23b, for each well in EPA's data template (see Table 7-2 for required elements). The required entries are for the following columns:
    a. **TYPE** should be one of the following codes: S0-S5 for standards; KC, NC, or SC for controls; P (primary) or D (for duplicate) of unknown sample.

b. **CONC** contains the numeric concentration value. Two special cases:
   i. Non-detected concentrations: If the sample is non-detected, provide the result in CONC column, record the data as 'ND' in the DATA FLAG column and provide the sample-specific detection limit (0.1 µg/L if the sample is undiluted with salinity less than 3.5 ppt) in the method detection limit column (MDL). See step 24 for the detection and reporting limit values for samples with salinity greater than or equal to 3.5 ppt. See **Section 7.7.6** for calculating the sample-specific detection limit for a diluted sample..
   ii. If the result shows that it is HIGH this indicates that the sample value is outside of the calibration range and must be diluted and re-run using another analytical run. Leave the CONC column blank and record 'HI' in the DATA FLAG column.

c. **DATA FLAGS** have codes for the following special cases:
   i. **ND** if the sample was non-detected;
   ii. **J** if the value is detected but at a level below the reporting limit of 0.15 µg/L (for undiluted samples);
   iii. **HI** if the concentration value registers as HIGH (exceeds the calibration range).

d. **QUALITY FLAGS** have codes for the following special cases:
   i. **QCF** if there is a QC failure per step 23 above. The QCF code must be used for all failures to facilitate data analysis.
   ii. **H** if the sample did not meet the holding time and was not analyzed within 90 days of collection.
   iii. **Q** for any other quality issue (describe in **COMMENTS**)

e. **DILUTION FACTOR** is only required if the sample was diluted.

f. **DUP AVG** and **DUP CV** are required for duplicate samples and control samples (use all three values if the controls are used in triplicate).

Table 7-2. Microcystins indicator: required data elements – data submission.

STAGE	FIELD	FORMAT	DESCRIPTION	
LOGIN	LAB ID	Text	Name or abbreviation for QC laboratory	
	DATE RECEIVED	Text	Date sample was received by lab	
	SITE ID	text	NWCA site ID code as recorded on sample label or tracking form (blank if standard or control)	
	VISIT NUMBER	numeric	Sequential visits to site (1 or 2) (blank if standard or control)	
	SAMPLE ID	numeric	6-digit Sample ID number as recorded on sample jar or tracking form (blank if standard or control)	
	DATE COLLECTED	MMDDYY	Date sample was collected (blank if standard or control)	
	CONDITION CODE	text	Sample condition upon arrival at the laboratory (blank if standard or control)	
			Flag	**Definition**
			Blank or N	Not a sample (blank, standard, or control)
			OK	Sample is in good condition
			C	Sample container is cracked
			L	Sample or container is leaking
			ML	Sample label is missing
			NF	Sample is not frozen
			Q	Other quality concerns, not identified above

STAGE	FIELD	FORMAT	DESCRIPTION	
	CONDITION COMMENT	text	Comments about the condition of the sample.	
ANALYSIS	BATCH ID	Numeric	Batch identification code, assigned by lab	
	TECHNICIAN	text	Name or initials of technician performing the procedure	
	DATE_ANALYZED	MMDDYY		
	KIT EXPIRE DATE	MMDDYY	Expiration date on kit box	
	KIT ID	text	Kit identification code. If one does not exist, assign a unique code to each kit.	
	R2	numeric	R^2 from curve fit to the average absorbance values for the standards. Value is between 0 and 1.	
	TYPE	text	Type of solution being tested in the well	
			Code	**Definition**
			KC	Kit Control
			NC	Negative Control
			S0, S1, S2, S3, S4, S5	Standard
			QC	QC sample
			U	Sample of unknown concentration
	LOCATION	text	Location of well in the kit (e.g., B5 would be the fifth well from the left in the second row B)	
	PRIM_DUP	text	Primary or duplicate run of a sample of unknown concentration (see **Figure 7-1**)	
	SALINITY	numeric	Conductivity values will be provided from NWCA HQ Team. Labs will be responsible for converting to salinity values and record the value in units of parts per thousand.	
	CONC	numeric	Concentration or sample-specific detection limit of contents of well in µg/L. Sample-specific detection limit should be 0.1 µg/L for a sample with salinity <3.5 ppt which hasn't been diluted. (Sample-specific detection limit is 0.175 µg/L for samples with salinity ≥3.5 ppt using the ADDA ELISA test kit).	
	UNITS	text	The units of the concentration of the CONC column	
	MDL	numeric	Minimum detection limit in the same units as the CONC column	
	RL	numeric	Reporting Limit in same units as the CONC column	
	ABSORBANCE	numeric	Absorbance value	
	DILUTION FACTOR	numeric	10, 100, etc for number of times the sample was diluted. If not diluted, leave blank or record 1	
	CV_ABSORB	numeric	Calculated %CV of duplicate values of absorbance for a sample. Only calculated for TYPE=U, KC, or NC. Enter %CV. Value is between 0 and 100%.	
	AVG_ABSORB	numeric	Calculated average of absorbance values for a sample. Only provided for TYPE=U, KC, NC, or SC. Average value of the original sample and its duplicate (or replicates for KC and NC).	
	AVG_CONC	numeric	Calculated average of concentration values for a sample.	

<table>
<tr><th>STAGE</th><th>FIELD</th><th>FORMAT</th><th colspan="2">DESCRIPTION</th></tr>
<tr><td></td><td>QA FLAG (if appropriate)</td><td>text</td><td colspan="2">Data qualifier codes associated with specific identifications of voucher samples. These codes provide more information that those used when reporting receipt of samples. A technician may use alternative or additional qualifiers if definitions are provided as part of the submitted data package (e.g., as a separate worksheet page of the data submission file).</td></tr>
<tr><td></td><td></td><td></td><td>Flag</td><td>Definition</td></tr>
<tr><td></td><td></td><td></td><td>ND</td><td>Concentration below detection. Unless the sample was diluted, the concentration will be 0.1 µg/L</td></tr>
<tr><td></td><td></td><td></td><td>H</td><td>Sample did not meet the holding time and was not analyzed within 90 days.</td></tr>
<tr><td></td><td></td><td></td><td>HI</td><td>Result indicated that a high concentration (i.e., outside calibration range)</td></tr>
<tr><td></td><td></td><td></td><td>J</td><td>Concentration above detection but below reporting limit.</td></tr>
<tr><td></td><td></td><td></td><td>QCF</td><td>QC failure</td></tr>
<tr><td></td><td></td><td></td><td>Q</td><td>Other quality concerns, not identified above</td></tr>
<tr><td></td><td>LAB COMMENTS</td><td>text</td><td colspan="2">Explanation for data flag(s) (if needed) or other comments.</td></tr>
</table>

7.7.5 Data Reporting Requirements

See **Table 7-3** for data reporting criteria.

Table 7-3. Microcystins indicator: data reporting criteria.

Measurement	Units	No. Significant Figures	Maximum No. Decimal Places
Microcystins	ug/L	3	3

7.7.6 Dilutions (if needed)

Dilutions if needed are prepared as follows (using clean glass tubes):

- 1:10 dilution
  a. Add 900 µL of distilled water to a clean vial. (Note: Dilutions may also be made using the kit's diluent rather than distilled water.)
  b. Pipette 100 µL from the sample into the vial. (To provide more accurate dilutions and less chance of contaminating the diluent, the diluent should be added to the vial before the sample.)
  c. Mix by vortexing.
  d. Multiply final concentration and Gold Standard Diagnostic's detection limit of 0.1 µg/L by 10 to obtain the sample-specific detection limit of 1.0 µg/L.
- 1:100 dilution

a. Add 3.96 mL of distilled water to a clean, appropriately labeled glass vial. (Note: Dilutions may also be made using the kit's diluent rather than distilled water.)

b. Vortex the sample to mix thoroughly, then pipette 40 µL from the sample and add to the water (or diluent) in the appropriate labeled vial. Vortex.

c. Multiply the final concentration and Gold Standard Diagnostic's detection limit of 0.1 µg/L by 100 to obtain the sample-specific detection limit of 10 µg/L.

- Other dilutions can be calculated in the same manner as #1 and #2 if needed.

7.8 Quality Measures

This section describes the QA/QC measures used to ensure that the data will meet NWCA requirements.

7.8.1 QC Samples

The Project Manager and the NWCA QA Team may decide to provide one or two identical sets of freshwater and/or seawater performance test samples to all participating laboratories. If the laboratory will assay both freshwater and seawater samples, then it will receive both sets (i.e., freshwater and seawater). Each set will contain up to five samples to test the expected range of concentrations in the NWCA samples.

For the contract laboratory, the first set of test samples will be run with the first set of samples and a second set will be run at the midpoint of the assigned samples. Because most state laboratories will have relatively few samples that can be analyzed using a single kit, only one set of test samples will be sent to each state laboratory.

Each laboratory will run the QC samples following the same procedures used for the other samples. The NWCA QA Team, or a delegate, will compare the results and assess patterns in the data (e.g., one laboratory being consistently higher or lower than all others). Based upon the evaluation, additional information may be requested from one or more laboratories about any deviations from the Method or unique laboratory practices that might account for differences between the laboratory and others. With this additional information, the NWCA QA Team will determine an appropriate course of action, including no action, flagging the data, or excluding some or all of the laboratory's data.

7.8.2 Summary of QA/QC Requirements

Table 7-4 provides a summary of sample receipt and processing QC, **Table 7-5** outlines the data quality objectives for microcystins and **Table 7-6** provides a summary of the sample analysis QC.

Table 7-4. Microcystins indicator: sample receipt and processing QC.

QC Activity	Description and Requirements	Corrective Actions
Demonstrate competency for analyzing microcystin samples to meet the	Demonstration of past experience with microcystin samples in achieving the method detection limits.	EPA will not approve any laboratory for NWCA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can

performance measures		demonstrate competency for its NWCA samples.
Sample Log-in	Upon receipt of a sample shipment, record receipt of samples in the NARS IM system (within 24 clock hours) and the LIMS.	Discrepancies, damaged, or missing samples are reported to the EPA HQs Laboratory QA Coordinator
Sample condition upon receipt	Sample issues such as cracked container; missing label; temperature (frozen); adherence to holding time requirements; sufficient volume for test.	Qualify samples
Sample Storage	Store sample frozen	Qualify samples
Holding time	Frozen samples can be stored for several months.	Qualify samples

Table 7-5. Microcystins indicator: data quality objectives.

Parameter	Units	Method Detection Limit Objective	Reporting Limit Objective
Microcystins, undiluted samples with salinities <3.5 ppt	µg/L	0.1	0.15
Microcystins, undiluted samples with salinity greater than or equal to 3.5 ppt	µg/L	0.175	0.263
Microcystins, diluted samples with salinities <3.5 ppt	µg/L	0.1 times the dilution factor	Will vary
Microcystins, diluted samples with salinity greater than or equal to 3.5 ppt	µg/L	1.75 times the dilution factor	Will vary

Table 7-6. Microcystins indicator: sample analysis QC activities and objectives.

QC Activity	Description and Requirements	Corrective Action
Kit – Shelf Life	Is within its expiration date listed on kit box.	If kit has expired, then discard or set aside for training activities.
Kit – Contents	All required contents must be present and in acceptable condition. This is important because Abraxis has calibrated the standards and reagents separately for each kit.	If any bottles are missing or damaged, discard the kit.

QC Activity	Description and Requirements	Corrective Action
Calibration	All of the following must be met: ○ Standard curve must have a correlation coefficient of ≥0.99; ○ Average absorbance value, $\bar{A}_0$, for S0 must be ≥0.80; and ○ Standards S0-S5 must have decreasing average absorbance values. That is, if $\bar{A}_i$ is the average of the absorbance values for S_i, then the absorbance average values must be: $\bar{A}_0 > \bar{A}_1 > \bar{A}_2 > \bar{A}_3 > \bar{A}_4 > \bar{A}_5$	If any requirement fails results from the analytical run are not reported. • All samples in the analytical run are reanalyzed until calibration provides acceptable results. • At its discretion, the lab may consult with EPA for guidance on persistent difficulties with calibration.
Kit Control	The average concentration value of the duplicates (or triplicate) must be within the range of 0.75 +/- 0.185 µg/L. That is, results must be between 0.565 and 0.935.	If either requirement fails: • Results from the analytical run are not reported • The lab evaluates its processes, and if appropriate, modifies its processes to correct possible contamination or other problems. • The lab reanalyzes all samples in the analytical run until the controls meet the requirements.
Negative Control	The values for the negative control replicates must meet the following requirements: ○ All concentration values must be below the reporting limit (i.e. <0.15 for undiluted sampled with salinities below 3.5 ppt) and ○ One or more concentration results must be nondetectable (i.e., <0.10 µg/L for undiluted sampled with salinities below 3.5 ppt)	
Sample Evaluations	All samples are run in duplicate. Each duplicate pair must have %Calibration Verification (CV) ≤15% between its absorbance values.	If %CV of the absorbances for the sample>15%, then: • Record the results for both duplicates. • Report the data for both duplicate results as Quality Control Failure (QCF); and • Re-analyze the sample in a new analytical run. **No samples are to be run more than twice.** If the second run passes, then the data analyst will exclude the data from the first run (which will have been flagged with QCF). If both runs fail, the data analyst will determine if either value should be used in the analysis (e.g., it might be acceptable to use data if the CV is just slightly over 15%).

QC Activity	Description and Requirements	Corrective Action
Results Within Calibration Range	All samples are run in duplicate. If both values are less than the upper calibration range (i.e., ≤ 5.0 µg/L for undiluted samples with salinity<3.5 ppt; ≤ 8.75 µg/L for undiluted samples with salinity ≥3.5 ppt), then the requirement is met.	If a result registers as "HIGH", then record the result with a data flag of "HI." If one or both duplicates register as 'HIGH,' then the sample must be diluted and re-run until both results are within the calibration range. **No samples are to be run more than twice.** If samples are re-run, do not enter concentration information of the first run.
External QC Sample	External QC Coordinator, supported by QC contractor, provides 1-2 sets of identical samples to all laboratories and compares results.	Based upon the evaluation, the External QC Coordinator may request additional information from one or more laboratories about any deviations from the Method or unique laboratory practices that might account for differences between the laboratory and others. With this additional information, the External QC Coordinator will determine an appropriate course of action, including no action, flagging the data, or excluding some or all of the laboratory's data.

7.9 Sample and Record Retention

The laboratory shall retain:

- The sample materials, including vials, for a minimum of 3 years from the date the EPA publishes the final report. During this time, the laboratory shall freeze the materials. The laboratory shall periodically check the sample materials for degradation.
- Original records, including laboratory notebooks and the reference library, for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

7.10 References

Gold Standard Diagnostics, "Microcystins-ADDA SAES ELISA (Microtiter Plate): Product No. 520011SAES" Retrieved on October 1, 2025 from https://www.goldstandarddiagnostics.com/microcystins-nodularins-adda-saes-elisa-96- tests.html

Gold Standard Diagnostics, “Microcystins in Brackish Water or Seawater Sample Preparation” Undated. Retrieved on October 1, 2025 from https://www.goldstandarddiagnostics.us/media/17276/tb-22-056-rev-01-microcystins-in-brackish-water-or-seawater-elisa.pdf

https://www.eurofins-technologies.com/microcystins-nodularins-adda-saes-elisa-96-tests.htmlJames, R., et al., “Environmental Technology Verification Report: Abraxis Microcystin Test Kits: ADDA ELISA Test Kit; DM ELISA Test Kit; Strip Test Kit,” in Environmental Technology Verification System Center 2010. Retrieved October 1, 2025 from http://nepis.epa.gov/Adobe/PDF/P100EL6B.pdf

8.0 RESEARCH INDICATOR: SOIL ISOTOPES

Soil isotopes laboratory procedures are not included in this manual. EPA CORAL scientists will process and analyze these samples in accordance with QA requirements established under the project's research plan.

APPENDIX A: DATA REPORTING TEMPLATES

Electronic reporting templates will be provided on EPA's NARS SharePoint site.

NWCA 2026 Plant ID_Lab Spreadsheets.xlsx
NWCA 2026 Water Chem-WCHL_Lab Spreadsheet.xlsx
NWCA 2026 Microcystins Lab Spreadsheet.xlsx
NWCA 2026 Soil Analysis Data Template.xlsx

APPENDIX B: SUPPLEMENTARY MATERIAL FOR VEGETATION – LISTS OF FLORISTIC RESOURCES

Accurate plant identification in the field or lab requires the use of local or regional floras and field guides appropriate to the area being sampled. It is also helpful to consult species range maps or distribution information to confirm species identifications. This appendix provides a list of floristic resources pertinent to individual states or to larger regions within the conterminous United States and Guam.

List of Regional, State, and Local Floristic Resources

EPA compiled a list of floras, field guides, and databases referenced in the NWCA 2026 FOM Appendix C, as a resource for NWCA Botanist/Ecologists. The full list is available electronically in spreadsheet format. Note: this list is not a complete compilation of floras and field guides available across the conterminous US and Guam. Rather, it includes floristic resources, applicable to specific states or regions, that were recommended by NWCA Field Crew Botanists/Ecologists. In addition, some other useful floristic resources referenced by herbaria or regional botanical expects are included on the list. Printed and online floras not included in this list (particularly recently published work) may also be considered for use during NWCA sampling.

Floristic Resources referenced in NWCA 2026 FOM Appendix C and provided as an electronic spreadsheet, are listed under three major subheadings: 1) resources covering the conterminous United States and Guam, 2) resources covering large regions (with a list of states to which they apply), and 3) resources that are applicable to specific individual states. The following information is provided for each resource: a Short Citation, a Full Citation, Resource Type, and Resource Applicability in the region or state. In addition, notes about the resource are provided where needed. The Short Citation is used to document the floristic resources used at each NWCA site for plant identification (see Recording Citations, below).

Recording Citations for Floristic Resources Used at NWCA Sampled Sites

The nomenclatural standard for the NWCA is the USDA-NRCS PLANTS Database (https://plants.usda.gov/), and all plant names will be reconciled to the PLANTS nomenclature during data preparation to facilitate data analysis. To aid in this process, it is important to know the floristic resources used for identification of plants at each sampled site.

The NWCA Veg Team will record short citations for the floristic resources used at each sampled site in the NWCA Data App on Form V-1 under the 'Floristic Resources Used' heading. There is space for up to eight resources to be included. The primary floristic resource used at the site should be listed first. A list of the short citations from the Appendix C are available in a drop-down list next to each citation entry space on Form V-1 to facilitate consistent data entry.

APPENDIX C: SUPPLEMENTARY MATERIAL FOR VEGETATION - PLANT PRESSING AND MOUNTING

Plant specimens are pressed and dried in a standard plant press (30 X 45 cm, 12 X 18 inches) composed of a breathable wooden frame, corrugated cardboard ventilators, blotters, folded newsprint, and a set of adjustable straps.

- The wooden frame and straps bound the press.
- Newsprint specimen folders, each containing plant material, are sandwiched between two moisture-absorbing blotters.
- The "blotter-newsprint sandwiches" are placed between corrugated cardboards.
- The corrugations of the cardboard should run parallel to the shorter dimension (30 cm) of the press for best air circulation. Bulky specimens may require extra blotters and cardboard.

Protocol for Pressing Plant Specimens:

1. To begin pressing a specimen, place a cardboard on the bottom wooden frame of the press, then add a blotter.
2. Lay a newsprint folder on top of the blotter. The newsprint folder should be affixed with a completed adhesive Plant Specimen Label. This label includes Site ID, the Plant Sample ID Number, and other critical data about the specimen you are pressing (see below for a complete list).
3. Clean as much dirt as possible off the plant material before placing it in the newsprint folder. Place the plant material inside the sheet of folded newsprint so that it lies entirely within the dimensions of the plant press.
4. Carefully arrange the plant material to display diagnostic features.
    - Lay the specimen flat and avoid overlapping plant parts.
    - Spread leaves, flowers, and fruits so they can be easily observed from different perspectives.
    - Show upper and lower surfaces of leaves and flowers.
    - If possible, arrange material so some flowers have the blossom open, and some flowers and fruits appear in longitudinal and transverse views.
    - Multiples of smaller plants of the same species should be pressed together on one sheet.
    - For large specimens, bend stems sharply into a V or N shape so they fit within the press frame. Avoid curving or twisting stems.
    - Thick stems, large fruits, or bulbs may be trimmed to reduce bulk by cutting them in half lengthwise.
5. Examples of small, loose plant parts (i.e., seeds, *Carex* perigynia) should be placed in a small paper packet or envelope inside of the newspaper.
6. Once the plant material is arranged, fold the newsprint closed.
7. Add another blotter, then a cardboard on top of the newsprint folder.

8. To begin pressing the next specimen, place a blotter over the top cardboard in the stack. Repeat steps 2 - 8 until the press is full or all specimens are included.
9. Use two adjustable straps to tighten and firmly compress the plant press and its contents.

Plant Specimen Label Information

- **Specimen Type:** Samples collected for QA purposes will have QA marked, while unknown samples will have Unknown marked.
- **Plant Sample ID Number:** Samples are assigned a plant collection identification number using the Site ID, the specimen type (unknown specimens are prefaced with the letter U and QA specimens are prefaced with the letter Q), and the row number the plant is recorded on in the data form. For example, the identification number for an unknown specimen recorded on row 14 at NWCA26-AL-10001 would be NWCA26-AL-10001-U14.
- **Collection Date:** Date is numerical: month, day, year, e.g. 06/14/2026.
- **County and State**: Information on county and state where specimen was collected.
- **Species Name or Pseudonym:** Species name from data form if known or descriptive name used on data forms if unknown (e.g., Carex sp. 1).
- **Collector(s) Name(s):** Lists the first name, middle initial and surname of the person or persons who collected the sample.
- **Abundance of Plant**: Indicates whether the species is dominant, common, sparse or uncommon at the site.
- **Type of Plant**: Describes growth form of the plant (graminoid, forb, shrub, tree, vine, or other).
- **Root Type**: Describes the type of roots found on the plant (taproot, fibrous roots, rhizomes, tubers, or other)
- **Water depth/Hydroperiod**: Indicates the soil moisture or water depth that the plant was collected from during sampling (dry, moist, top of hummock, in 6 inches of water, perennially saturated, etc.).
- **Sunlight**: Describes the average amount of sun exposure the plant can be found growing in (full sun, part sun, part shade, or full shade).
- **Growth Habit**: Describes detailed characteristics of stem growth (erect, arching, trailing, shrubby, or other).
- **Number of Petals and Flower Color**: Describes characteristics of the flower as they appear when collected.
- **Habitat**: The type of plant community or setting where the plant is growing (e.g., wetland type (Cowardin, HGM, NVC), wetland community type (forested wetland, emergent marsh, wet prairie, mountain bog, etc.), anthropogenic disturbances (urban setting type), and other plants growing in association (associated species information would be available from the plot)).
- **Multiple Newsprints**: Fill this circle in if more than one newsprint is needed to preserve a specimen. In “Newsprint ___ of ___”, number each individual newsprint accordingly (e.g., 1 of 3).

Drying Plant Specimens

Pressed plant specimens should be removed from the presses once they are thoroughly dried.

- To encourage drying, keep full presses in a warm, dry, well-ventilated location in the vehicle during the day and in a well-ventilated warm location at the lodging location or the laboratory/herbarium at night.

- As the specimens dry, they will lose volume, so periodically tighten the straps on the press to maintain pressure on the specimens and minimize shrinkage and wrinkling.
- Rapid and thorough drying is enhanced by low humidity and ample airflow around and through the presses. The best preservation of color and morphology is obtained with rapid drying over low heat. Also, dry air circulating through the press may kill many insects and insect eggs, potentially protecting the specimens from damage.
- The easiest way to achieve these conditions is by using an electric plant dryer that provides steady bottom heat (95°F to 113°F), where plants usually dry in 12 to 48 hours. Plant dryers are typically constructed as a simple box with a heat source (often light bulbs) and a fan for air circulation, on which plant presses can be placed to accelerate drying.
- Once plant specimens are dry, remove them from the presses with individual specimens kept in their newsprint folders with attached Plant Specimen Labels.

Mounting Plant Samples

Vascular plants should:

- Be mounted on archival-quality paper measuring 11.5 X 16.5 inches
- Be mounted using commercially available acid-free adhesive, such as polyvinyl acetate (PVA)
- Allow for placement of a properly filled out Plant Specimen Label
- Have an acid-free fragment envelope (where necessary for seeds and flowers)

APPENDIX D: LABORATORY REMOTE EVALUATION FORMS

Email the completed and signed forms to Kendra Forde (forde.kendra@epa.gov).

Questions: Contact Kendra Forde at forde.kendra@epa.gov or 202-566-0417

NWCA 2026 DOCUMENT REQUEST FORM

In 2026, EPA and its State and Tribal partners will conduct the next National Wetland Condition Assessment. NWCA is a survey of the nation's wetlands. It is designed to provide statistically valid regional and national estimates of the condition of wetlands. Consistent sampling and analytical procedures ensure that the results can be compared across the country.

As part of the NWCA 2026, the NWCA QA Team has been requested to conduct a technical assessment to verify QC practices in your laboratory and its ability to perform analyses under this project. Our review will be assessing your laboratory's ability to receive, store, prepare, analyze, and report sample data generated under EPA's NWCA 2026.

The first step of this assessment process will involve the review of your laboratory's certification and/or documentation. Subsequent actions may include (if needed): reconciliation exercises and/or a site visit.

All laboratories will be required to complete and submit the following checklist and appropriate signature form, along with accompanying documentation, for the specific indicator(s) for which your laboratory will be conducting analysis for the NWCA 2026.

Contents

NWCA 2026 General Laboratory Request Form

Laboratory Name_____

Which samples are you analyzing?

- ☐ Water chemistry analytes identified in the LOM and QAPP including chlorophyll-*a*
- ☐ Microcystins
- ☐ Soils
- ☐ Vegetation (Vouchers/Unknowns)

If your lab has been previously approved for the work under NARS for the specific indicator(s) listed above within the last 5 years:

- ☐ A *signature* on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for labs conducting analyses for the 2026 NWCA.
- ☐ A signature on the Quality Assurance Project Plan (QAPP) indicates that you will follow both the QAPP and the LOM.

For previously approved laboratories, please submit the following to the EPA HQ NWCA Laboratory Review Coordinator if available:

- ☐ Documentation of a successful *quality assurance evaluation* from a prior National Aquatic Resource Survey (NARS) that occurred within the last 5 years.
- ☐ Documentation showing participation in a previous NARS **for the specific indicator(s) listed above** for the same parameters/methods.

If you have not been approved within the last 5 years through the laboratory quality assurance evaluation for the indicator(s) or you do not have the documentation listed above, we request that you submit the following documents (if available) for review so EPA can determine your ability to participate as a laboratory in the NWCA (if none of these are available, please contact Kendra Forde, the EPA HQ NWCA Laboratory Review Coordinator to discuss alternative documentation).

- ☐ A copy of your laboratory's *accreditations and certifications* if applicable (i.e. NELAC, ISO, state certifications, NABS, etc.).
- ☐ An updated copy of your laboratory's *QAPP* and Laboratory Quality Assurance Manuals
- ☐ *Standard Operating Procedures* (SOPs) for your laboratory for each analysis to be performed (if not covered in NWCA 2026 LOM).
- ☐ Documentation attesting to experience analyzing the NWCA 2026 indicators identified above (e.g., water chemistry for NWCA includes a number of parameters including chlorophyll-*a)*.
- ☐ Documentation of qualifying credentials in plant taxonomy for the *botanists* (if applicable).

Laboratory Signature Form – Chemistry Laboratories

I,_____certify that the laboratory,_____
_____located in _____, will abide by the following standards in performing the following data analysis and reporting for the 2026 National Wetland Condition Assessment (NWCA).

This applies to the _____ chemistry indicator(s).

1.) Use procedures identified in the NWCA 2026 Laboratory Operations Manual (LOM), or equivalent. If using equivalent procedures, please provide the procedures and obtain approval from EPA.
2.) Read and abide by the NWCA 2026 Quality Assurance Project Plan (QAPP) and LOM.
3.) Have an organized IT tracking system in place for recording sample tracking and analysis data.
4.) Provide data using the template provided on the NARS SharePoint site.
5.) Provide data results in a timely manner. This will vary with the type of analysis and the number of samples to be processed. Sample data must be received no later than March 30, 2027 or as otherwise negotiated with EPA.
6.) Participate in a laboratory technical assessment if requested by EPA NWCA staff (this may be a conference call or on-site assistance visit).
7.) Agree to analyze for all parameters specified in the LOM for the appropriate indicator(s) identified above, including Chlorophyll-a, for Water Chemistry.

Signature _____ Date _____

National Wetland Condition Assessment 2026: Vegetation Laboratory Quality Assurance Evaluation

The National Wetland Condition Assessment (NWCA) is designed to provide statistically valid regional and national estimates of the condition of wetlands in the 48 conterminous states of the U.S. Plant samples collected in the field are sent to a designated laboratory/herbarium for identification using standard laboratory protocols outlined in the NWCA 2026 Laboratory Operations Manual (LOM).

As specified in the NWCA Quality Assurance Project Plan (QAPP), a NWCA Evaluator will evaluate each laboratory/herbarium to ensure the NWCA data quality objectives are satisfied. **Each laboratory/herbarium must participate in an evaluation and sign the laboratory signature form and acknowledgement and commitment to implement page of the QAPP to satisfy the terms of the NWCA QAPP.**

It is essential that each laboratory/herbarium accurately implement standardized protocols for vegetation identification and storage to ensure comparability of data among NWCA sites and minimize data loss that could result from damaged or degraded specimens, errors in data recording, sample processing, data storage, plant identification, or misinterpretation of guidance for laboratory operations. These quality assurance evaluations are designed to:

1. Confirm the NWCA 2026 Laboratory Operations Manual (LOM) protocols are implemented as intended.
2. Assist with questions the laboratory/herbarium may have.
3. Suggest corrections if any errors have been made by a laboratory/herbarium in implementing methods described in the LOM.

This evaluation will include a discussion of the attached checklist between the NWCA Evaluator and the laboratory/herbarium over the phone rather than an actual laboratory visit. The checklist includes descriptions of sample handling and other requirements to which each laboratory/herbarium must comply. The discussions will be scheduled by the Laboratory Review Coordinator or their delegate.

Background: For all NWCA field work, whenever the identity of a species cannot be confirmed in the field, a sample is collected for later identification in the office by the field botanist/ecologist or by another botanist at a designated laboratory/herbarium. All unknown species located in one of five Vegetation Plots arrayed across a site's Assessment Area that are mature and have key structures needed for identification are collected (unknown species voucher). Unknown species that are immature or senescent comprising more than 5% cover are also collected. The field botanist/ecologist will ship unknown samples they cannot identify to the botanist (also called plant ID specialist or taxonomist in NWCA) at the laboratory/herbarium for initial identification.

In addition to all unknown specimens, field crews collect three known plant voucher samples (randomly selected from species identified by the Vegetation Team) for quality assurance (NWCA 2026 QAPP). These QA vouchers are sent to a QA "verifying botanist" for re-identification/verification. Collecting voucher specimens of known species both provides a quality assurance check on species identity data, and a permanent record of the occurrence of a particular species at a given location.

The QA verifying botanist is responsible for re-identification/verification of the QA vouchers as well as a random selection of 10% of the unknown specimens that were initially determined by the "identifying botanist" at the laboratory/herbarium.

If the unknown species specimens and QA voucher samples are planned to be sent to the same institution, it is important that all quality assurance activities be completed by a taxonomist that did not participate in the identification of unknown specimens.

All laboratory methods and quality assurance requirements are fully described in the NWCA 2026 LOM and QAPP.

For the purposes of the Vegetation Laboratory Quality Assurance Evaluations, the Vegetation Checklist will focus on the lab's competence to receive and properly store specimens and to track and manage the plant taxa data.

Definitions:

Voucher Sample - A pressed and dried plant sample, ideally comprised of leaves, stems, flowers, fruits and roots. An integral component of each voucher sample is written data describing the location, date of collection, habitat, plant habit, characteristic features and other information. Vouchers provide physical evidence that confirms the presence of plant species at specific locations.

Identifying Botanist - The person identifying and processing unknown samples. This could be a field botanist/ecologist; university, state, national or regional herbarium botanist; or an EPA contractor that has qualifying credentials in plant taxonomy. The identifying botanist is responsible for ensuring all plant identification and processing tasks outlined in the LOM are completed. In some cases, this may require the identifying botanist to identify partners to assist with the work.

QA Verifying Botanist – The person re-identifying and verifying QA voucher identifications and a 10% subset of unknown species identifications by the laboratory/herbarium. This could be a botanist, ecologist, taxonomist, and/or plant ID specialist that is an expert in the identification of wetland plants. The verifying botanist agrees to use the NWCA prescribed methods, as described in Section 4 of the LOM, to ensure that all QA vouchers are correctly verified.

Laboratory/herbarium - represents the person or entity identifying and processing unknown samples. This could be a field botanist/ecologist, state identified herbarium, EPA identified regional herbarium, or National EPA Contractor. The Laboratory/herbarium is responsible for ensuring all plant identification and processing tasks outlined in NWCA manuals (LOM and QAPP) are completed. In some cases, this may require identifying partners to assist with the work.

VEGETATION LABORATORY QUALITY ASSURANCE EVALUATIONS
NWCA 2026 VEGETATION LABORATORY ASSISTANCE CHECKLIST

Lab ID: _____ Individuals Performing IDs: _____

Individuals on Conference Call: _____

Vegetation Lab Evaluator: _____

Date: _____

Instructions:

1. All vegetation laboratories must adhere to methods and QA requirements described in the NWCA 2026 Laboratory Operations Manual (LOM) and the NWCA Quality Assurance Project Plan (QAPP).
2. The Vegetation Laboratory Evaluator will discuss the following subjects with the vegetation laboratories/herbarium and circle the data bubble that reflects the results of their observation.
3. Yes = the task is being correctly completed, No = the task is not being completed as described, or Not Applicable = the task was not needed at this lab.

Supplies and Equipment for Sample Handling			
Checklist:	**Y**	**N**	**N/A**
Does the vegetation lab/herbarium have the following supplies and equipment?			
• Plant dryer			
• Dissecting microscope			
• Herbarium cabinet or sealable plastic boxes for storing dried plant samples prior to identification			
• Dissecting tools (e.g., single edge razor blades, forceps, dissecting needles)			
• Regional floras and plant lists (identify all floras the lab plans to use for NWCA project):			
• Access to USDA-NRCS PLANTS standardized taxonomic nomenclature (https://plants.usda.gov/)			
• *2026_NWCA Plant ID_Lab Spreadsheets.xlsx* data template			
• Plant sample tracking forms			
• Plant sample folders			

• Freezer for freeze treating plant specimens for pests, or other approved method. If other method is used, describe below:			
• OPTIONAL: Mounting materials (herbarium sheets, mounting glue, forceps, weights for holding samples with wet glue to the herbarium receiving voucher samples)			
• OPTIONAL: Herbarium sample labels			
Notes:			

Processing and Managing Plant Samples			
Plant samples may arrive at the vegetation lab/herbarium as: 1) dried, pressed samples, or 2) pressed but still wet plant material enclosed in a plant press.			
Drying Samples:	**Y**	**N**	**N/A**
• If samples arrive in a press, but still wet, are the samples placed on a plant dryer to complete drying, and then be treated for pests?			
• Does the lab place the full presses on an electric plant dryer that provides steady bottom heat (95°F to 113°F), for plants to dry in 12 to 48 hours?			
• If a plant dryer is not available, are the presses placed in a warm, dry location to allow drying? Does the lab provide a place for low ambient humidity and good airflow around and through the presses to ensure rapid and thorough drying of plant material?			
• If plants arrive in a press, does the lab periodically tighten the straps on the press to maintain pressure on the samples and minimize shrinkage and wrinkling until plant identification?			
Treating Samples for Detritivores, Molds, and Pests: *Dried plant material is highly susceptible to contamination by detritivores, molds, and pests that can destroy herbaria collections; therefore, it is important to treat all incoming samples to kill potential contaminants.*	**Y**	**N**	**N/A**
• Does the lab have standard treatment procedures for pests and are those procedures being implemented for the NWCA specimens?			
• After the samples are pressed and dried, does the lab treat them for detritivores, molds, and pests?			

• If freezing, is the lab freezing the samples at (-20°C or below) for at least three days for loosely stacked samples and seven days for tightly packed samples?			
Storing Samples:	**Y**	**N**	**N/A**
• Are plant samples being stored in herbarium cabinets or sealable plastic containers when not in use?			
• Does the lab ensure that samples are not left out in the herbarium room overnight? If samples are found to have been left out overnight or if an herbarium cabinet/plastic container has been left open, does the lab decontaminate all samples again?			
Notes:			
Tracking Specimens – Tracking Forms T-4 and T-5, Plant Sample Specimen Label			
Tracking Forms: *In the field, each voucher sample collected is assigned a set of tracking information, which is recorded on the Plant Sample Tracking Forms (Form T-4: Unknown Plant Shipment and T-5: QA Plant Shipment). It is important that every specimen sent to and received by the lab is tracked following the protocols described in* ***Section 4.2.2*** *of the LOM.*	**Y**	**N**	**N/A**
• Does the lab review all the Tracking Forms to ensure that all samples listed are received by the lab?			
• If a sample listed on the tracking form is not part of the shipment received, does the lab **contact the NARS Information Management Coordinator (541-754-4793; gover.michelle@epa.gov) as soon as possible?**			
Plant Specimen Label: *Every sample will arrive at the laboratory/herbarium with a Plant Specimen Label. This label includes diagnostic information for known and unknown species collected.*	**Y**	**N**	**N/A**
• Does the lab review the information provided on the Plant Specimen Label included with the sample? ○ Plant Sample ID and Collection Number ○ Date ○ Visit Number ○ County and State of Site ○ Scientific Name or Pseudonym ○ Collector(s) Name(s) ○ Abundance of Plant ○ Type of Plant ○ Root Type ○ Water depth/Hydroperiod for Plant at Sampling ○ Sunlight			

○ Growth Habit ○ Number of Petals and Flower Color ○ Habitat			
Notes:			
Identification of Vegetation Samples			
Names for all NWCA plants specimens identified (unknowns) or verified (QA vouchers) need to be reconciled to the standard found in USDA-NRCS PLANTS (https://plants.usda.gov/).	**Y**	**N**	**N/A**
• Is the lab reconciling names for all species that they identify to the standard found in USDA-NRCS PLANTS?			
• Is the lab a Heritage program or coordinating with a Heritage program or university herbarium? Name (if yes): _____			
• Is the lab using a reference herbarium and is it the state's reference herbarium? Name (if yes): _____ **check box if state reference herbarium**			
• How many unknown plant samples does the lab identify in a typical year?	# of samples: _____		
Notes:			
Mounting and Storing Herbarium Sheets			
Once the samples are dried, pressed, and identified, they are to be stored at the vegetation lab/herbarium for at least five years.	**Y**	**N**	**N/A**
• Does the lab have the storage capabilities and facilities to properly store dried, pressed, and identified samples for at least five years?			
• Will vouchers be kept in sealable plastic containers in a cool dry climate and will they be accessible to the EPA?			
• Will the lab incorporate the NWCA vouchers into their permanent collections?			
• Will vouchers from the national survey be mounted on herbarium sheets and labeled to indicate that they were collected as part of the NWCA?			

Notes:			
Sending Resultant Data Forms			
Data must be reported to EPA electronically using the 2026_NWCA Plant ID_Lab Spreadsheets.xlsx *data template. The template includes separate spreadsheets for recording names of identified unknowns and reconciling to USDA-NRCS PLANTS nomenclature, and for recording re-identification and verification of QA voucher specimens.*	**Y**	**N**	**N/A**
• The lab is aware of these reporting requirements and is sending in the resultant data per the instructions in *2026_NWCA Plant ID_Lab Spreadsheets.xlsx* data templates.			
• The species identifications are regularly entered into the appropriate spreadsheets, and these forms are transmitted at appropriate intervals to the HQ Project Management Team.			
Notes:			
Quality Assurance/Quality Control			
A subset of plant samples collected as unknown specimens and later identified by the lab will need to be verified by a verifying botanist for additional quality assurance. The lab will randomly select 10% of the identified unknown samples to be sent to the verifying botanist, another experienced botanist, taxonomist, and/or plant ID specialist who did not participate in the original identifications. A chain-of-custody form (Tracking Form T-4: Unknown Plant Shipment) needs to be completed and sent with the specimens.	**Y**	**N**	**N/A**
• If the field botanist/ecologist is acting as the laboratory/herbarium for unknown plant voucher identifications, does the lab ensure that another qualified botanist, or a state or EPA identified laboratory/herbarium is used for QA?			
• Does the lab ensure that the person who made the first identification of the unknown sample is not the same person making the second identification of the sample (i.e., ten percent of all “unknown species”)?			
• Does the lab record all identifications in the *2026_NWCA Plant ID_Lab Spreadsheets.xlsx* for each sample, and email to the HQ Project Management Team?			
Notes:			

Laboratory Signature Form – Vegetation Laboratory/Herbarium

I, _____, certify that the laboratory/herbarium, _____ _____ located in _____, will abide by the following standards in processing and reporting plant taxa data for the National Wetland Condition Assessment 2026 (NWCA).

1.) Use procedures identified in the NWCA 2026 Laboratory Operations Manual (LOM), or equivalent. Any departure from these procedures must obtain prior approval from EPA.

2.) Read and abide by the NWCA 2026 Quality Assurance Project Plan (QAPP) and LOM.

3.) Have an organized IT tracking system in place for recording sample tracking and analysis data.

4.) Notify HQ Project Management Team of any substantial differences in taxonomic identifications between the identifying botanist(s) and the verifying botanist(s).

5.) Provide data using the template provided on the NARS SharePoint. Contact the EPA Laboratory Review Coordinator for a copy or for questions.

6.) Provide data results in a timely manner. This will vary with the type of analysis and the number of samples to be processed. Sample data must be received no later than December 15, 2026 or as otherwise negotiated with EPA.

7.) Participate in a laboratory technical assessment if requested by EPA NWCA staff (this may be a conference call or on-site assistance visit).

Signature _____ Date _____