United States Environmental Protection Agency
Office of Water
Washington, DC
EPA 843-R-15-009

National Wetland Condition Assessment 2016

Laboratory Operations Manual

Version 1.1, April 2016

NOTICE

The objective of the National Wetland Condition Assessment 2016 (NWCA 2016) project is to describe the ecological condition of the nation's wetlands and stressors commonly associated with poor condition. The complete documentation of overall project management, design, methods, quality assurance, and standards is contained in four companion documents:

National Wetland Condition Assessment 2016: Field Operations Manual – 843-R-15-007
National Wetland Condition Assessment 2016: Quality Assurance Project Plan – 843-R-15-008
National Wetland Condition Assessment 2016: Laboratory Operations Manual – 843-R-15-009
National Wetland Condition Assessment 2016: Site Evaluation Guidelines – 843-R-15-010

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 2016. All Project Cooperator laboratories should follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. 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. 2015. *National Wetland Condition Assessment 2016: Laboratory Operations Manual*. EPA-843-R-15-009. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

VERSION HISTORY

Version	Date	Changes Made
1.0	2/12	n/a
1.1	4/18	Updated table 6.4 by adding column of acceptable reporting limits for NWCA water chemistry and chlorophyll-a parameters; updated figures for revised tracking forms; corrected minor grammatical errors and typos

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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
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	
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
PQL	
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) 2016 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 contains procedures for laboratory analysis of samples collected from wetlands throughout the conterminous 48 states of the United States. The purposes of this manual are to:

1) document the standardized sample processing and analysis procedures used in the various laboratories for the NWCA 2016
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: algal toxins (microcystins), soils, water chemistry and chlorophyll a, and vegetation. It should be noted that specific laboratory analysis procedures for water chemistry 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. 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 project management team (EPA Office of Water).

2.0 GENERAL LABORATORY GUIDELINES

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 Quality Assurance Project Plan (QAPP).

2.2 Roles and Contact Information

The **EPA Headquarters (HQ) Project Management Team** consists of the Project Manager, Alternate Project Manager, NARS QA Lead, Logistics Lead, and Laboratory Review Coordinator. The Team is responsible for overseeing all aspects of the project and ensuring technical and quality assurance requirements are properly carried out. The Team is the final authority on all decisions regarding laboratory analysis.

The **NARS Information Management (IM) Coordinator** tracks the location of each NWCA 2016 sample that involves post-processing. The coordinator will be the labs main point of contact in regards to sample tracking and data submission.

Table 2-1 Contact information

Title	Name	Contact Information
EPA HQ NWCA Project Manager	Gregg Serenbetz, OW	serenbetz.gregg@epa.gov 202-566-1253
EPA HQ NWCA Alternate Project Manager	Chris Faulkner, OW	Faulkner.chris@epa.gov 202-566-1185
EPA HQ NARS QA Lead	Sarah Lehmann, OW	lehmann.sarah@epa.gov 202-566-1379
EPA HQ Logistics Lead	Colleen Mason, OW	Mason.colleen@epa.gov 202-343-9641
EPA HQ NWCA Laboratory Review Coordinator	Kendra Forde, OW	kendra.forde@epa.gov 202-564-0417
NARS IM Coordinator	Marlys Cappaert, SRA International Inc.	cappaert.marlys@epa.gov 541-754-4467

2.3 Sample Tracking

Samples are collected by a large number of different 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 electronic forms when they have shipped samples and the NARS IM Center will input each sample into the NARS IM database. Laboratories can track sample shipment from field crews by accessing the NARS IM database. Participating laboratories will be given access to the NARS IM system, where they can acquire tracking numbers and information on samples that have been shipped

to them by field crews (either by overnight shipment for perishable samples or batch shipments for preserved samples). Upon sample receipt, the laboratory must immediately log in to the database and confirm that samples have arrived. Overnight samples may not be loaded into the database prior to sample arrival, but should be tracked by the laboratory and receipt information inputted into the database when sample information is loaded. Each lab will make arrangements with the NARS IM Coordinator, listed above, to ensure access is granted.

When the samples arrive from the field crews, laboratories should also receive tracking forms in the shipment (refer to the NWCA 2016 FOM). These forms will list the samples that should be included in the shipment. Laboratory personnel should cross check the forms with the samples received to verify that there are not any inconsistencies. If any sample is missing or damaged, contact the NARS IM Coordinator immediately.

2.4 Reporting

All laboratories must provide data analysis information to the HQ Project Management Team and the NARS IM Center by **March 30, 2017** or as stipulated in contractual agreements. These reports must include the following information:

- Sample Type (indicator)
- Site ID (ex: NWCA16-1001)
- Sample ID (ex: 999000)
- Pertinent information to the indicator
- Metadata for all fields

See Appendix C 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., 2016_11_01)
- Lab name (e.g., MyLab)

Combined, the file name would look as follows: WaterChemistry_2016_11_01_MyLab.xlsx

As specified in the QAPP, remaining sample material and specimens must be maintained by the EPA's designated laboratory or facilities as directed by the NWCA 2016 Project Lead. All samples and raw data files (including logbooks, bench sheets, and instrument tracings) are to be retained by the laboratory for 3 years or until authorized for disposal, in writing, by the EPA Project Lead. Deliverables from contractors and cooperators, including raw data, are permanent as per EPA Record Schedule 258. EPA's project records are scheduled 501 and are also permanent.

3.0 LABORATORY QUALITY CONTROL

As part of the NWCA 2016, 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 2016 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 2016.

Given the large number of labs participating in the NWCA 2016, it is not feasible to perform an assistance visit[1] (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 EPA Headquarters Project Management Team will conduct remote review of lab certifications and accreditations of all labs. This process is called laboratory verification. If issues arise from the remote review that cannot be resolved remotely then an on-site visit to the lab will be performed. The NWCA 2016 Project Management Team believes this approach meets the needs of this assessment and can ensure quality control 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, the laboratory shall provide analyte and matrix 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:

- 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.
- Demonstration of competency with sediment samples in achieving the method detection limits, accuracy, and precision targets.

Quality assurance and quality control requirements.

To demonstrate its competency in quality assurance and quality control 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 Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NWCA QAPP Certification Page.

1 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 as “inspection” as in a traditional audit.

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 2016 LOM. Laboratory evaluation reinforces the specific 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 2016 project, we have requested that each participating laboratory provide the following documentation:

- The laboratory's Quality Manual, Quality Management Plan or similar document
- Standard Operating Procedures (SOPs) for each analysis to be performed
- Long term Method Detection Limits (MDLs) for each instrument used and Demonstration of Capability for each analysis to be performed
- A list of the laboratory's accreditations and certifications, if any
- 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 audit 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 review and evaluation of the documentation requested.

If a laboratory meets or exceeds all of 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 is in need of help or fails to produce the requested documentation.

All labs must sign the NWCA 2016 QAPP signature page. In addition, all labs must sign a Lab Signature Form (Appendix B) indicating that they will abide by the following:

1. Utilize procedures identified in the NWCA 2016 LOM (or equivalent). If using equivalent procedures, please provide procedures manual to demonstrate ability to meet the required minimum quality objectives (MQO).

2. Read and abide by the NWCA 2016 Quality Assurance Project Plan (QAPP) and related Standard Operating Procedures (SOP).
3. Have an organized IT system in place for recording sample tracking and analysis data.
4. Provide data using the template referenced in the LOM.
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 May 1, 2017 or as otherwise negotiated with EPA.
6. Participate in a lab technical assessment or audit if requested by EPA NWCA staff (this may be a conference call or on-site audit).

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

1. Use taxonomic standards outlined in the NWCA 2016 LOM.

4.0 VEGETATION

4.1 Introduction

Wetland plant species 1) represent diverse adaptations, ecological tolerances, and life history strategies, and 2) effectively integrate environmental conditions, species interactions, and human-caused disturbance. Data describing plant species composition and abundance and vegetation structure are powerful, robust, and relatively easy to gather. They can be used to derive myriad 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 in 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. 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.

For NWCA, crews will collect unknown plant specimens ("unknown species vouchers") and known plant species for quality assurance purposes ("QA vouchers") from each site and send to a designated laboratory/herbarium for identification.

4.2 Receiving Voucher Samples

Plant samples will arrive at the laboratory/herbarium pressed in shipping boxes. 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 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. 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 Botanist/Ecologist will ship unknown samples to the **identifying botanist** at the laboratory/herbarium for initial identification (Vegetation Chapter of FOM).

For the purposes of this manual, the identifying botanist represents 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 this manual are completed. In some cases this may require lab partners to assist with the work.

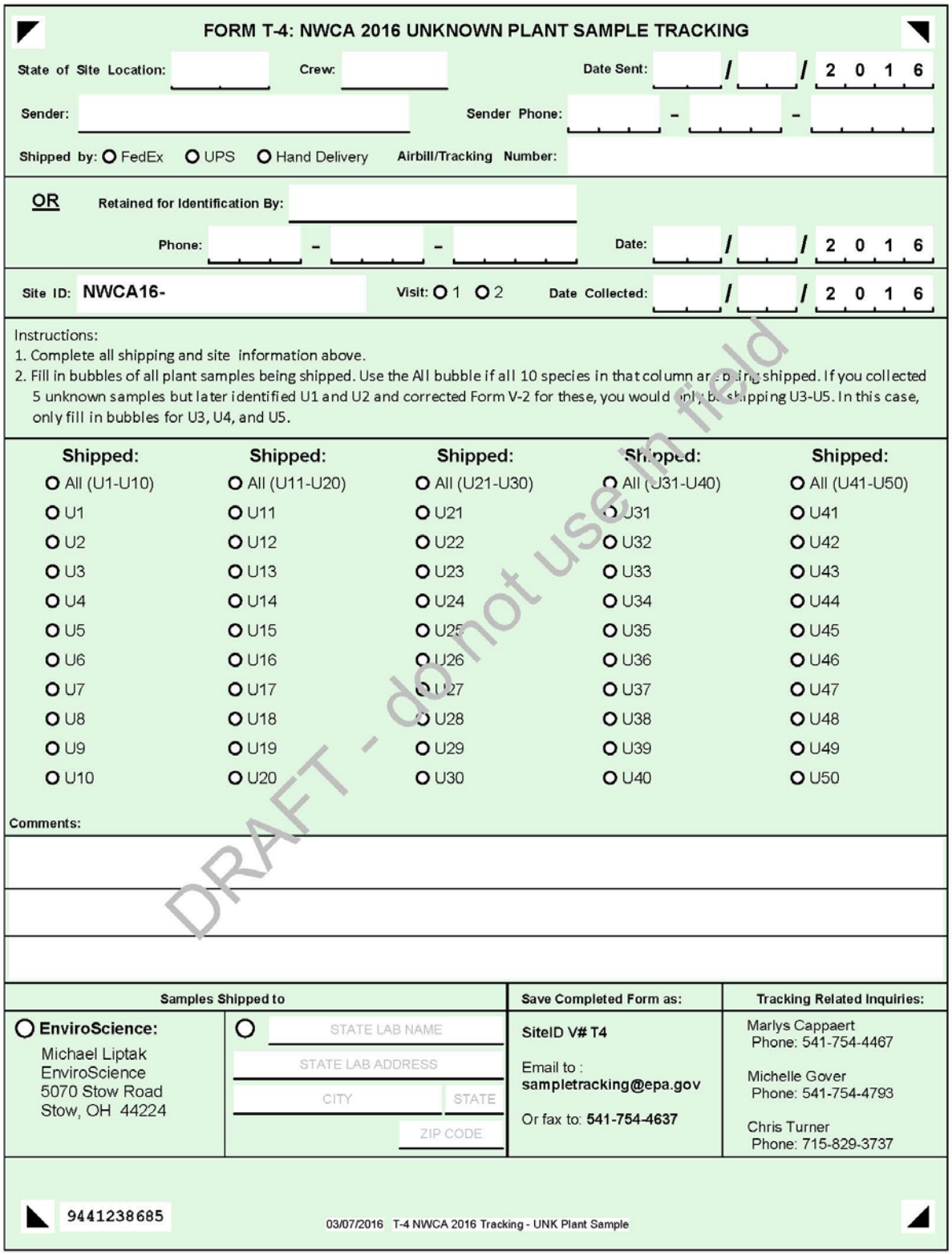
In addition to all unknown specimens, field crews will be submitting five known plant voucher samples (randomly selected from species identified by the Vegetation Team) for quality assurance (NWCA 2016 QAPP). These **QA voucher** specimens will be sent to a QA **verifying botanist** for re-identification/verification (Vegetation Chapter of FOM). Collecting voucher samples 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 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 all quality assurance activities be completed by a taxonomist that did not participate in the identification of unknown specimens. .

4.2.2 Tracking information

In the field, each voucher sample collected is assigned a set of tracking information, which is recorded on the Plant Sample Tracking Forms (Figures 4-1 and 4-2). At the end of the sampling week, the Vegetation Team will remove the samples and newspaper sleeves from the press, ensuring they retain the Plant Specimen Label (Figure 4-3), and ship them in a sturdy box to either the identifying botanist for unknown samples or the QA verifying botanist for the five known specimens (Vegetation Chapter of FOM). 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 EPA Project Management Team immediately.



FORM T-4: NWCA 2016 UNKNOWN PLANT SAMPLE TRACKING

State of Site Location: ____ Crew: ____ Date Sent: ____ / ____ / 2016

Sender: ____ Sender Phone: ____ - ____ - ____

Shipped by: O FedEx O UPS O Hand Delivery Airbill/Tracking Number: ____

OR Retained for Identification By: ____

Phone: ____ - ____ - ____ Date: ____ / ____ / 2016

Site ID: NWCA16- Visit: O 1 O 2 Date Collected: ____ / ____ / 2016

Instructions:
1. Complete all shipping and site information above.
2. Fill in bubbles of all plant samples being shipped. Use the All bubble if all 10 species in that column are being shipped. If you collected 5 unknown samples but later identified U1 and U2 and corrected Form V-2 for these, you would only be shipping U3-U5. In this case, only fill in bubbles for U3, U4, and U5.

Shipped:	Shipped:	Shipped:	Shipped:	Shipped:
O All (U1-U10)	O All (U11-U20)	O All (U21-U30)	O All (U31-U40)	O All (U41-U50)
O U1	O U11	O U21	O U31	O U41
O U2	O U12	O U22	O U32	O U42
O U3	O U13	O U23	O U33	O U43
O U4	O U14	O U24	O U34	O U44
O U5	O U15	O U25	O U35	O U45
O U6	O U16	O U26	O U36	O U46
O U7	O U17	O U27	O U37	O U47
O U8	O U18	O U28	O U38	O U48
O U9	O U19	O U29	O U39	O U49
O U10	O U20	O U30	O U40	O U50

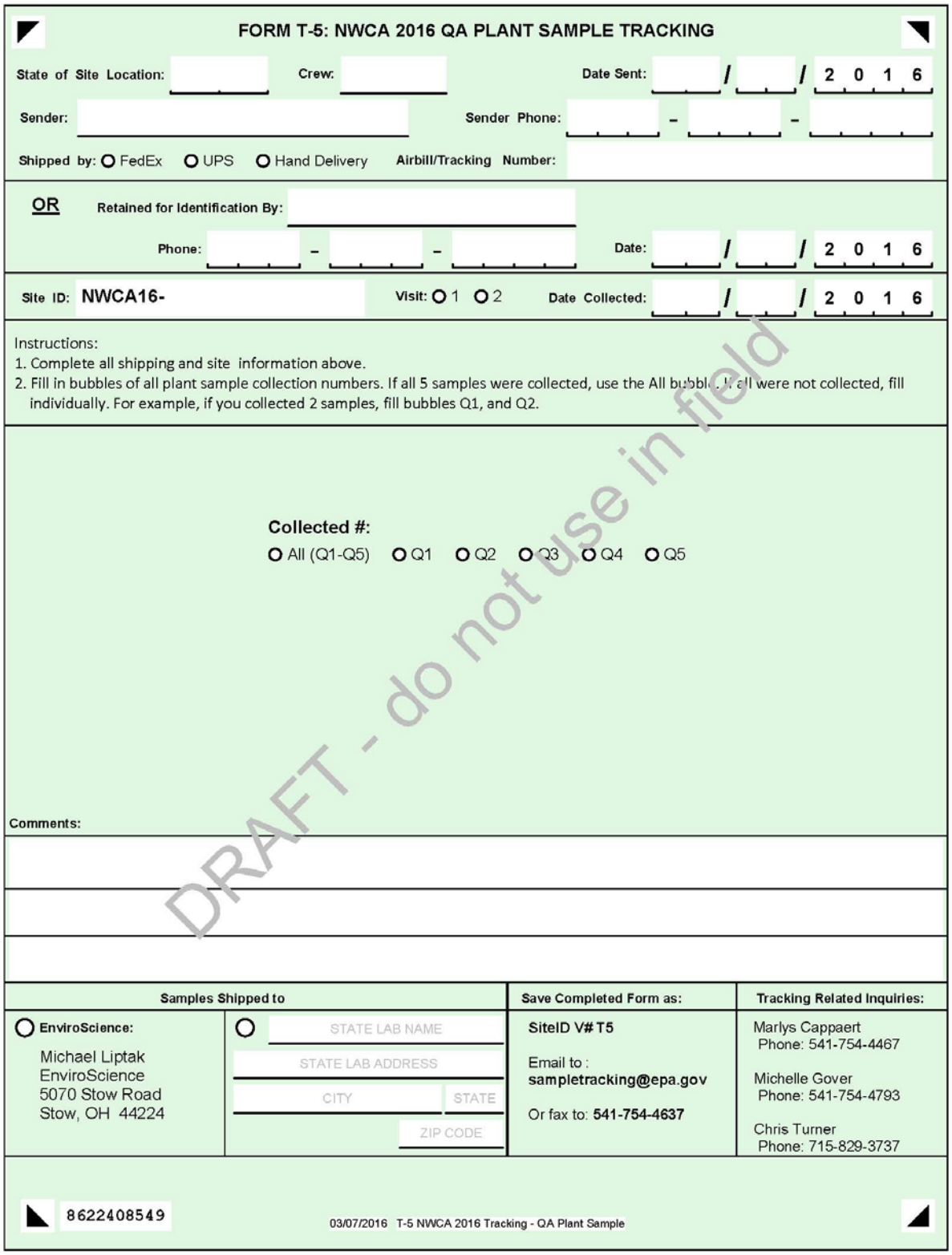
Comments:

DRAFT - do not use in field

Samples Shipped to		Save Completed Form as:	Tracking Related Inquiries:
O EnviroScience: Michael Liptak EnviroScience 5070 Stow Road Stow, OH 44224	O STATE LAB NAME STATE LAB ADDRESS CITY STATE ZIP CODE	SiteID V# T4 Email to : sampletracking@epa.gov Or fax to: 541-754-4637	Marlys Cappaert Phone: 541-754-4467 Michelle Gover Phone: 541-754-4793 Chris Turner Phone: 715-829-3737

9441238685 03/07/2016 T-4 NWCA 2016 Tracking - UNK Plant Sample

Figure 4-1. Unknown Plant Sample Tracking Form



FORM T-5: NWCA 2016 QA PLANT SAMPLE TRACKING

State of Site Location: Crew: Date Sent: / / 2016

Sender: Sender Phone: - -

Shipped by: O FedEx O UPS O Hand Delivery Airbill/Tracking Number:

OR Retained for Identification By:

Phone: - - Date: / / 2016

Site ID: NWCA16- Visit: O 1 O 2 Date Collected: / / 2016

Instructions:
1. Complete all shipping and site information above.
2. Fill in bubbles of all plant sample collection numbers. If all 5 samples were collected, use the All bubble. If all were not collected, fill individually. For example, if you collected 2 samples, fill bubbles Q1, and Q2.

Collected #:
O All (Q1-Q5) O Q1 O Q2 O Q3 O Q4 O Q5

DRAFT - do not use in field

Comments:

Samples Shipped to		Save Completed Form as:	Tracking Related Inquiries:
O EnviroScience: Michael Liptak EnviroScience 5070 Stow Road Stow, OH 44224	O STATE LAB NAME STATE LAB ADDRESS CITY STATE ZIP CODE	SiteID V# T5 Email to : sampletracking@epa.gov Or fax to: 541-754-4637	Marlys Cappaert Phone: 541-754-4467 Michelle Gover Phone: 541-754-4793 Chris Turner Phone: 715-829-3737

8622408549 03/07/2016 T-5 NWCA 2016 Tracking - QA Plant Sample

Figure 4-2. QA Plant Sample Tracking Form

4.3 Supplies and Equipment for Sample Handling

- Plant dryer
- Dissecting microscope
- Dissecting tools (e.g., single edge razor blades, forceps, dissecting needles)
- Regional floras and plant lists
- USDA PLANTS taxonomic standard http://plants.usda.gov/java/
- Plant nomenclatural forms
- Plant sample tracking forms
- Plant sample folders
- Storage cabinet or sealable plastic boxes for storing dried plant samples prior to identification
- OPTIONAL: Freezer or 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 laboratory/herbarium in several 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 pests.

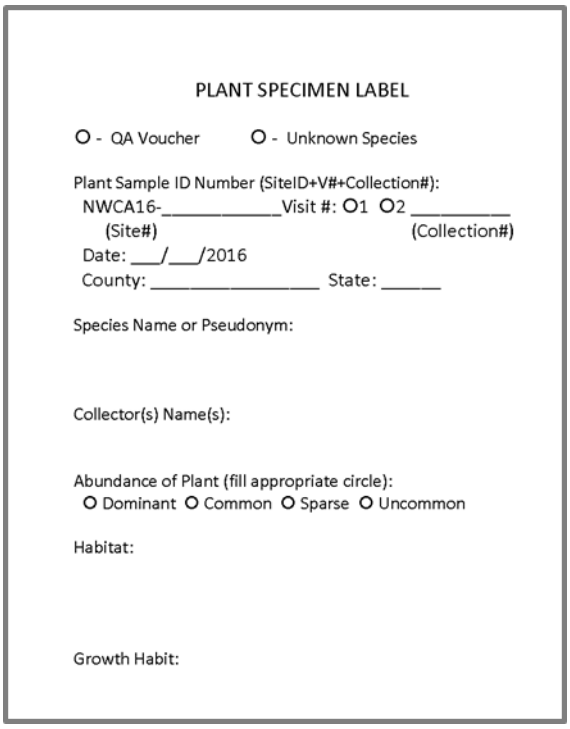
4.4.1 Plant Sample Label Form

Every sample will arrive with a Plant Specimen Label. This label includes the original identification and diagnostic information for known and unknown species collected including location, date of collection, habitat, plant habit, and abundance information. Voucher samples are considered incomplete without this information. An example of the Plant Specimen Label is provided in Figure 4-3. If a sample does not have any of the following information, contact the EPA Project Management Team immediately:

Plant Specimen Label Information

- **Specimen Type:** Samples collected for QA purposes will have QA Voucher filled in, while unknown samples will have Unknown Species filled in.
- **Plant Sample ID Number:** NWCA Site Number-Plant collection number. Plant collection numbers for samples are assigned consecutive numbers depending on the specimen type (unknown specimens are prefaced with the letter U and QA specimens are prefaced with the letter Q) for each site beginning with one. For example, the sample number for the 14th unknown specimen collected at NWCA16-9999 would be NWCA16-9999-U14.
- **Visit Number:** Indicates whether it was the first visit (1) or a repeat visit (2). Most sites are only visited once.
- **Collection Date:** Date is numerical: month, day, year, e.g. 06/14/2016.

- **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 (e.g., Carex sp. 1) if unknown.
- **Collector(s) name:** 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.
- **Habitat**: The type of plant community or setting where the plant is growing. (e.g., such as 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).
- **Growth habit**: Describes key features of the plant such as growth form (tree, shrub, vine, herb), approximate height, longevity (annual, biennial, perennial), clonal, rhizomatous, tussock-forming, etc. Lists any characteristics of the plant which may be lost upon drying, such as flower/fruit color, fragrance, and leaf orientation.


PLANT SPECIMEN LABEL

O - QA Voucher O - Unknown Species

Plant Sample ID Number (SiteID+V#+Collection#):
NWCA16-_____Visit #: O1 O2 _____
(Site#) (Collection#)
Date: ___/___/2016
County: _____ State: _____

Species Name or Pseudonym:

Collector(s) Name(s):

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

Habitat:

Growth Habit:

Figure 4-3. Plant specimen label

4.4.2 Drying Samples

Plant samples may arrive wet and in the plant press. The pressed plants must be thoroughly dried before removing them from the presses. As the samples dry they will lose volume, so it is often necessary to periodically tighten the straps on the press to maintain pressure on the samples and minimize shrinkage and wrinkling.

Low ambient humidity and good airflow around and through the presses 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 some 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 them (-20°C or below) for at least three days for loosely stacked samples and seven days for tightly packed samples.

To protect the collection from 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 that have been left out overnight or if a cabinet/plastic container has been left open, all samples may need to be decontaminated again.

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 is 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 the taxonomic nomenclature of the USDA Plants Database as its taxonomic standard. To effectively key plants and identify them in the field, however, field crews may use local floras appropriate to each region or state (Appendix D). This means numerous taxonomies will likely be applied across the 48 conterminous states comprising the study area. The identifying botanist will reconcile all species names to the standard found in USDA-NRCS PLANTS at http://plants.usda.gov/.

4.5.2 Recording Identifications

All identifications are recorded in an Excel database (2016 NWCA Plant ID Lab Spreadsheets). The Excel database 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 of 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 Excel spreadsheets for the QA voucher and Unknown specimen spreadsheets. Once the spreadsheets in the database have been completed, copies are then sent to the project facilitator (QAPP Section 5.1.6).

4.6 Mounting and Storing Herbarium Sheets

Once the samples are dried, pressed, and identified, they are to be stored at the 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 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 E.

4.7 Quality Assurance

A subset of plant samples collected as unknown specimens and later identified by a State or National Plant Laboratory botanist ("identifying botanist") will be verified by a QA taxonomist ("verifying botanist") for additional quality assurance. The lab will randomly select 10% of the identified unknown samples 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 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.

Quality control procedures associated with sample handling and processing at laboratories handling NWCA QA and unknown plant vouchers are summarized in Table 4-1.

Table 4-1. Laboratory quality control activities for vegetation indicator.

Quality Control Activity	Frequency	Acceptance Criteria	Corrective Action
Demonstrate competency for identifying samples to meet the performance measures	Once	Demonstration of past experience relevant to identifying plants collected from wetlands	EPA will not approve any laboratory for NWCA voucher identifications if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory 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.
Sample Log-in	All vouchers	Plant vouchers logged into NARS IM system within 24 clock hours of receipt.	Discrepancies, damaged or missing samples are reported to EPA Project Manager and Laboratory Review Coordinator.
Store sample 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 expects that the laboratory will exercise every effort to maintain vouchers in proper storage conditions.

Quality Control Activity	Frequency	Acceptance Criteria	Corrective Action
Use widely / commonly accepted taxonomic references and reconcile to USDA-NRCS PLANTS taxonomic nomenclature	All identifications	Full citations for floras and field guides used in plant identification must be provided and; identifications must be reconciled to the taxonomic nomenclature of the USDA-NRCS PLANTS database	Lab will provide explanation and discuss deviances with EPA TOCOR.
Identification by laboratory	When field plant ID specialist cannot identify specimen	Identification by lab plant ID specialist (who must be a different individual than the field plant ID specialist)	Replace field crew's "unknown" identification with determination by lab
Unknowns QC	Approximately 10% of all unknown vouchers independently identified in the lab	PTD ≤ 15%	If PTD > 15%, review data for possible explanations; otherwise, insert data qualifier for laboratory identifications
Conduct assistance visit	EPA may choose to visit any laboratory	Visit conducted using checklist	Performance and any recommended improvements described in debrief with laboratory 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. http://www.k-state.edu/herbarium/pests.html. Accessed November 17, 2009.

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 (CVS, http://cvs.bio.unc.edu) and the North Carolina Ecosystem Enhancement Program (EEP, http://www.nceep.net).

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.

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. 2006. Survey of the Nation's Lakes. Laboratory Methods Manual. EPA841-B-06-005. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2006. Survey of the Nation’s Lakes. Quality Assurance Project Plan. EPA841-B-07-003. 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.

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.

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

5.2 Summary of Method

Three types of soil samples will be collected from each site:

- Standardized Depth Soil Core – collected from the Soil Plot, represents a layer from the soil surface to the 10 cm depth. Analysis will include chemical parameters and particle size distribution (soil texture).
- Horizon Bulk Density Sample – collected from the Soil Pit, three samples are collected from all horizons greater than or equal to 8 cm thick to a depth of 1.0 m. Samples will be analyzed for bulk density.
- Horizon Chemistry Sample – collected from the Soil Pit, a sample is collected from every horizon to a depth of 1.0 m. Analysis will include chemical parameters and particle size distribution (soil texture).

The Standardized Depth Soil Core, Horizon Bulk Density Samples, and Horizon Chemistry Samples will be analyzed by the Kellogg Soil Survey Lab (KSSL), National Soil Survey Center (NSSL). A total of 13 analytical methods will be performed to characterize the soil chemical and physical properties (Table 5-1). Soil bulk density measurements will be made on Horizon Bulk Density Samples, all other parameters will be measured on the Standardized Soil Depth Core and the Horizon Chemistry Samples.

Table 5-1. Summary of NWCA 2016 soil analytical methods.

Analysis Method	Analyte(s) Measured
Particle Size Distribution Analysis (PSDA), < 2mm, air dry	Clay, Silt, Sand
Calcium Carbonate Equivalent, < 2mm	$CaCO_3$
Calcium Carbonate Equivalent, < 20 mm	$CaCO_3$
Total Carbon, Nitrogen, and Sulfur	C, N, S
pH	1:1 H_2O, 1:2 0.01 M $CaCl_2$
Cation Exchange Capacity and Base Cations	CEC, Ca^{2+}, K^+, Mg^{2+}, Na^+
Ammonium Oxalate Extraction	Al, Fe, Mn, P, Si
Electrical Conductivity	EC
Dithionite-Citrate Extraction	Al, Fe, Mn
Olsen Phosphorus	P

Analysis Method	Analyte(s) Measured
Mehlich Phosphorus	P
Trace Elements	Ag, As, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, P, Pb, Sb, Se, Sn, Sr, V, W, Zn
Bulk Density	Db_f

5.3 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions. Specific safety warnings and guidelines for each of the analytical methods can be found in the Kellogg Soil Survey Laboratory Methods Manual (Soil Survey Staff, 2014).

5.4 Sample Handling and Processing

5.4.1 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 (see example, USDA APHIS PPQ 525-A, Figure 5-1 below).

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 water proof 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.

[Placeholder for copy of APHIS permit (Figure 5-1) when received from NRCS]

5.4.2 Laboratory Sample Preparation

Each sample type collected is preassigned a six-digit site specific sample ID number. Samples are also labeled with Site ID, visit number, and horizon number. Laboratory identification numbers and preparation codes are assigned to each soil sample by the NRCS laboratory. These unique identification numbers carry important information about the soil sample (e.g., site, year sampled, soil horizon, replicate). Laboratory preparation codes depend on the properties of the sample and the requested analyses. Identification numbers and preparation codes are reported on the KSSL Primary Characterization Data Sheets. Refer to the Soil Survey Investigations Report No. 45, Soil Survey Laboratory Information Manual (Soil Survey Staff, 2011), for a detailed explanation of sample identification numbers. Detailed information on the current preparation codes as they appear on the Primary Characterization Data Sheets may be obtained from the KSSL upon request.

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 Kellogg Soil Survey Laboratory Methods Manual, Soil Survey Investigations Report No. 42, Version 5.0 (Soil Survey Staff, 2014).

5.5 Summary of Analytical Methods

Analytical methods used at the Kellogg Soil Survey Laboratory are summarized in Table 5-2. Method procedures are described in detail in the Kellogg Soil Survey Laboratory Methods Manual, Soil Survey Investigations Report No. 42, Version 5.0 (Soil Survey Staff, 2014). These are the standard operating procedures of the Lab, and are standard methods, peer-recognized methods, KSSL-developed methods, and/or methods specified in Keys to Soil Taxonomy (2014).

Table 5-2. NWCA 2016 soil analytical methods. Analyses follow the standard operating procedures of the Kellogg Soil Survey Lab (KSSL), National Soil Survey Center, Natural Resources Conservation Service.

Analyte	Method	Summary of Method	KSSL Method
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, >20 mm		4E1a1a1a2
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 atomic absorption spectrophotometer (AAS).	4B1a1a1a1 4B1a1b1-4
Al **Fe** **Mn** **P** **Si**	Ammonium Oxalate Extraction	Soil sample is extracted with a mechanical vacuum extractor in a 0.2 M ammonium oxalate solution buffered at pH 3.0 under darkness. The ammonium oxalate extract is weighed, diluted, and analytes are measured by an inductively coupled plasma atomic emission spectrophotometer (ICP-AES).	4G2a1a1-5
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

Analyte	Method	Summary of Method	KSSL Method
Al **Fe** **Mn**	Dithionite-Citrate Extraction	Soil sample is mixed with sodium dithionite, sodium citrate, RODI water, and shaken overnight; solution is centrifuged and extract is diluted; analytes are measured by an atomic absorption spectrophotometer (AAS).	4G1a1-3a-b1
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 **As** **Ba** **Be** **Cd** **Co** **Cr** **Cu** **Hg** **Mn** **Mo** **Ni** **P** **Pb** **Sb** **Se** **Sn** **Sr** **V** **W** **Zn**	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
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.	3B6a

Analyte	Method	Summary of Method	KSSL Method
		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).	

5.6 Quality Assurance / Quality Control (QA/QC) Procedures

Standardized lab protocols, consistent training of all lab technicians, lab assistance visits to all labs, and availability of experienced technical personnel to respond to site-specific questions as they arise are important to ensuring the quality of lab data. Additionally, control measures to minimize measurement error among lab technicians and laboratories include the use of laboratory quality control samples and a data review and validation process (QAPP Section 5.2.5).

5.6.1 Laboratory Performance Requirements

Table 4.3 summarizes the pertinent laboratory performance requirements for the soil indicators.

Table 5-3. Soil laboratory method performance requirements.

Analyte	Method	Units	MDL	PQL	Potential Sample Range[1]	Accuracy Objective
Clay **Silt** **Sand**	PSDA, <2 mm, air dry	%	na	na	0 to 93.1 0.1 to 100 0 to 94.5	
$CaCO_3$	Calcium Carbonate Equivalent, <2mm	%	0.5	2.5	nd to 105	
	Calcium Carbonate Equivalent, <20mm	%	0.5	2.5	nd to 96	
C **N** **S**	Total Carbon, Nitrogen, and Sulfur	%	0.04	0.2	nd to 62.43 nd to 11.193 nd to 21.86	0.01% 0.001% 0.01%
pH	1:1 H_2O	pH	na	na	2.4 to 10.5	0.1 pH unit
	1:2 0.01 M $CaCl_2$	pH	na	na	2.4 to 10.5	0.1 pH unit
CEC **Ca^{2+}** **K^{+}** **Mg^{2+}** **Na^{+}**	Cation Exchange Capacity by NH_4OAC, pH 7	cmol(+) kg^{-1}	0.1 0.07 0.06 0.01 0.2	0.6 0.4 0.3 0.07 1.0	nd to 252 nd to 507.3 nd to 17.4 nd to 147.1 nd to 650.3	0.1 cmol(+) kg^{-1} 0.1 cmol(+) kg^{-1} 0.1 cmol(+) kg^{-1} 0.1 cmol(+) kg^{-1} 0.1 cmol(+) kg^{-1}
Al **Fe** **Mn**	Ammonium Oxalate Extraction	% % mg kg^{-1}	0.002 0.0001 0.1	0.009 0.0006 0.6	nd to 15.62 nd to 20.15 nd to 15730.7	0.01% 0.01% 1.0 mg kg^{-1}

[1] nd = non-detect, tr = trace

Analyte	Method	Units	MDL	PQL	Potential Sample Range[1]	Accuracy Objective
P		mg kg^{-1}	26	129	nd to 16926.4	1.0 mg kg^{-1}
Si		%	0.0002	0.001	nd to 6.13	0.01%
EC	Electrical Conductivity	mmhos cm^{-1}	0.001	0.005	nd to 167.4	0.01 mmhos cm^{-1}
Al	Dithionite-Citrate Extraction	%	0.001	0.006	nd to 8.6	0.1%
Fe			0.01	0.07	nd to 36.6	0.1%
Mn			0.0006	0.003	nd to 3.6	0.1%
P (Olsen)	Olsen Extraction	mg kg^{-1}	0.1	0.7	nd to 399.7	0.1 mg kg^{-1}
P (Mehlich No. 3)	Mehlich No. 3 Extraction	mg kg^{-1}	0.1	0.6	nd to 1232.3	0.1 mg kg^{-1}
Ag	Trace Elements	mg kg^{-1}	0.001	0.01	nd to 175.62	
As		mg kg^{-1}	0.002	0.01	nd to 1808.06	
Ba		mg kg^{-1}	"0"	"0"	0.02 to 4415.8	
Be		mg kg^{-1}	0.001	0.01	nd to 29.98	
Cd		mg kg^{-1}	0.001	0.01	nd to 85.68	
Co		mg kg^{-1}	"0"	"0"	nd to 1125.58	
Cr		mg kg^{-1}	0.006	0.03	nd to 2020.31	
Cu		mg kg^{-1}	0.002	0.01	nd to 1036.28	
Hg		µg kg^{-1}	1.3	6.50	nd to 26060	
Mn		mg kg^{-1}	0.002	0.01	nd to 692942	
Mo		mg kg^{-1}	0.001	0.01	nd to 235.17	
Ni		mg kg^{-1}	0.009	0.05	tr to 3347.36	
P		mg kg^{-1}	0.4	2.07	nd to 70708.6	
Pb		mg kg^{-1}	0.001	0.01	nd to 12287.4	
Sb		mg kg^{-1}	0.002	0.01	nd to 42.01	
Se		µg kg^{-1}	1.8	9.00	nd to 16523.1	
Sn		mg kg^{-1}	0.005	0.03	nd to 1117.66	
Sr		mg kg^{-1}	0.001	0.01	nd to 10895	
V		mg kg^{-1}	"0"	"0"	nd to 1064.65	
W		mg kg^{-1}	"0"	"0"	nd to 137.39	
Zn		mg kg^{-1}	0.006	0.028	0.06 to 10379.1	
Bulk Density	Bulk Density Core Method	g cm^{-3}	na	na	0.15 to 2.6	0.01 g cm^{-3}

5.6.2 Laboratory Quality Control Samples

Laboratory quality control 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 four data reviews, first by the Bench Analysts, second by the Lead Analyst, third by the Project Coordinator Soil Scientist, and fourth by a Soil Scientist Liaison with expertise in soils from the region where the samples are from. The Bench Analysts verifies that blank and control samples return results that fall within established control limits. The Lead Analyst examines 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. The Project Coordinator will use professional judgment to 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. Data reviews include range checks, summary statistics, and/or exploratory data analysis. Identified reporting errors are corrected or data is qualified as suspect or invalid as appropriate. A final review is given by a Soil Scientist Liaison to the area of sample origin, before the data are released. Data reporting units and significant figures are given in Table 5-4. Indicator QC coordinator determines impact and possible limitations on overall usability of data based on the specific issue. The NWCA 2016 Project QA Officer is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members.

Table 5-4. Soil data reporting criteria.

Analyte	Method	Units	Number of Significant Figures	Maximum Number of Decimal Places
Clay	PSDA, <2 mm, air dry	%	3	1
Silt			3	1
Sand			3	1
$CaCO_3$	Calcium Carbonate Equivalent, <2mm	%	3	0
	Calcium Carbonate Equivalent, <20mm	%	3	0
C	Total Carbon, Nitrogen, and Sulfur	%	4	2
N			4	2
S			4	2
pH	1:1 H_2O	pH	2	1
	1:2 0.01 M $CaCl_2$	pH	2	1
CEC	Cation Exchange Capacity by NH_4OAC, pH 7	cmol(+) kg^{-1}	3	1
Ca^{2+}			4	1
K^+			3	1
Mg^{2+}			4	1
Na^+			4	1

Analyte	Method	Units	Number of Significant Figures	Maximum Number of Decimal Places
Al	Ammonium Oxalate Extraction	%	4	2
Fe		%	4	2
Mn		mg kg^{-1}	6	1
P		mg kg^{-1}	6	1
Si		%	3	2
EC	Electrical Conductivity	mmhos cm^{-1}	4	2
Al	Dithionite-Citrate Extraction	%	2	1
Fe			3	1
Mn			2	1
P (Olsen)	Olsen Extraction	mg kg^{-1}	4	1
P (Mehlich No. 3)	Mehlich No. 3 Extraction	mg kg^{-1}	5	1
Ag	Trace Elements	mg kg^{-1}	5	2
As		mg kg^{-1}	6	2
Ba		mg kg^{-1}	5	2
Be		mg kg^{-1}	4	2
Cd		mg kg^{-1}	4	2
Co		mg kg^{-1}	6	2
Cr		mg kg^{-1}	6	2
Cu		mg kg^{-1}	6	2
Hg		µg kg^{-1}	4	0
Mn		mg kg^{-1}	6	2
Mo		mg kg^{-1}	5	2
Ni		mg kg^{-1}	6	2
P		mg kg^{-1}	7	2
Pb		mg kg^{-1}	7	2
Sb		mg kg^{-1}	4	2
Se		µg kg^{-1}	7	2
Sn		mg kg^{-1}	6	2
Sr		mg kg^{-1}	7	2
V		mg kg^{-1}	6	2
W		mg kg^{-1}	5	2
Zn		mg kg^{-1}	7	2
Bulk Density	Bulk Density Core Method	g cm^{-3}	3	2

5.7 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. 2011. Soil Survey Laboratory Information Manual. Soil Survey Investigations Report No. 45, Version 2.0. R. Burt (ed.). U.S. Department of Agriculture, Natural Resources Conservation Service.

Soil Survey Staff. 2014. Kellogg Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42, Version 5.0. R. Burt and Soil Survey Staff (ed.). 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

This chapter describes the analysis requirements for water quality samples. The purpose is to determine concentrations of water quality parameters in water quality samples collected in the NWCA 2016. The laboratory shall perform analysis to determine levels of conductivity, pH, ammonia (NH_3), nitrate-nitrite (NO_3-NO_2), total nitrogen (TN), total phosphorous (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. Water chemistry parameters measured by NWCA 2016.

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

6.1 Summary of Method

As an alternative to specifying laboratory methods for sample analysis, NWCA 2016 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, unless otherwise contractually bound by other requirements, the laboratory may choose to use any method that meets EPA's specifications for water chemistry measurements.

6.2 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:

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 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.

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

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

6.3.1 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."

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 Injected 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. Typical values for the dilution factors will be 10 or 100.

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

SO_4: Sulfate

TN: Total nitrogen

TP: Total phosphorous

6.3.2 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 B for more information.

Quality assurance and quality control requirements. To demonstrate its expertise in quality assurance and quality control procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), Laboratory Quality Assurance Manuals, QAPPs, and applicable Standard Operating Procedures (SOPs). See Appendix B for more information.

To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NWCA QAPP Certification Page.

6.3.3 Personnel

The procedure refers to the following personnel:

Laboratory Technician: This procedure may be used by any laboratory technician who is familiar with the NWCA Quality Assurance Project Plan, and this procedure in the NWCA Laboratory Operations Manual.

6.3.4 Equipment/Materials

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

6.4 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 'CHLA'

The laboratory technician must inspect the samples promptly on receipt and:

1. Log the samples into the National Aquatic Resource Survey Information Management system (NARS-IM) within 24 clock hours. Alternatively, for shipments with a large number of samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARS-IM.
2. Check that each shipping container has arrived undamaged. Check 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 record the reading. Temperature of the wet ice shipments should be 4 ºC or at less. Record the condition and temperature of the sample in the database using the codes in Table 6-2.
3. Verify that all required data elements, per Table 6-2, have been recorded in the NARS IM database. If any data elements are missing, then enter them into the database.
4. Transfer 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 30 days before analysis. Except during processing and analysis stages, the filter must be stored frozen to less than or equal -20 °C ± 2°.
5. 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_COLLECT	Date	Date that the field crew collected the sample

Variable	Type	Description	
ANALYSIS_TYPE	Character	Water Chemistry (CHEM) or Chlorophyll *a* (CHLA)	
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	
		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)	

6.5 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 filtration.
2. Rinse vacuum filter funnel units thoroughly with reverse-osmosis (RO) or de-ionized (DI) water (ASTM Type II reagent water) five times before each use and in between samples. After placing a filter in the funnel unit, run approximately 100 mL of RO or DI 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 RO or DI water before continuing.
4. Split the sample into two aliquots as shown in Figure 6-1.
5. Add ultra-pure acid (H_2SO_4, depending on the analytes, see Table 6-3) to one of the two aliquots. Cap the bottle tightly and inverts 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, TN and DOC; and prepare one bottle without acid. Cap the bottle tightly and invert the bottle several times to mix.

2. Store all aliquots in a refrigerator at 4°C in darkness.

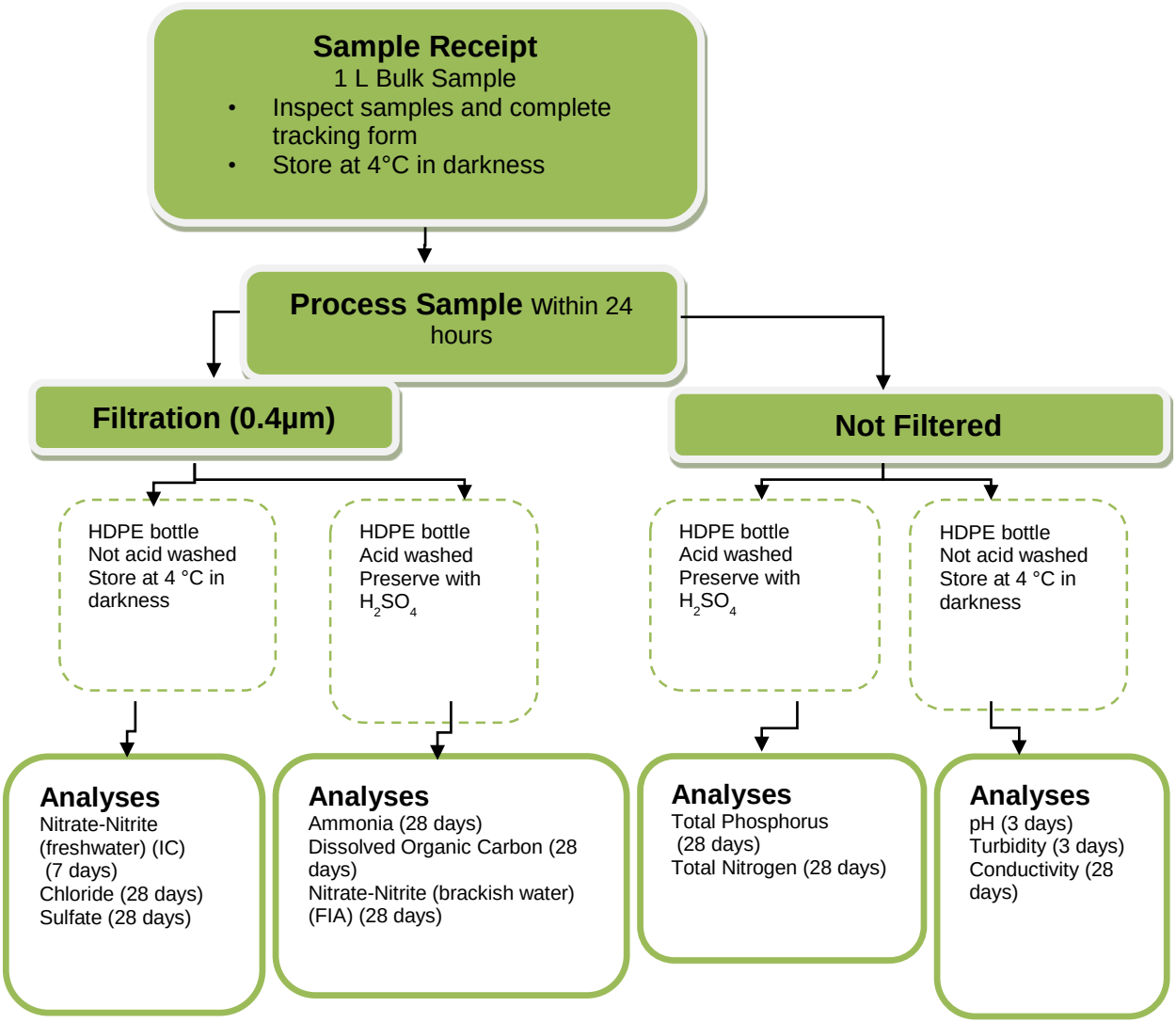


Figure 6-1. Water chemistry sample processing procedures

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_2-NO_3 (when FIA method used)

6.6 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 phosphorous (TP), dissolved organic carbon (DOC), conductivity, turbidity, pH, and chlorophyll *a*. We are considering the addition of sulfate and chlorides for freshwater samples. 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 the value of the lowest non-zero calibration standard that the laboratory must use. EPA has set the value to double the long-term method detection limit (LT-MDL), following guidance presented in USGS (1999)[1].

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.

For standard samples (of known concentration), 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. Accuracy is estimated as the difference between the mean measured value and the target value of a performance evaluation and/or internal reference samples at the lower concentration range measured across sample batches, and as the percent difference at the higher concentration range.

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 B identifies the information that the laboratory should

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

provide to the NWCA Laboratory Review Coordinator to use in determining whether the laboratories meet the necessary requirements.

Table 6-4 Water Chemistry and Chlorophyll-a: Laboratory Method Performance Requirements

Parameter	Units	Potential Range of Samples[1]	Method Detection Limit Objective[2]	Target Reporting Limit	Acceptable Reporting Limit	Transition Value[3]	Precision Objective[4]	Accuracy Objective[5]
Conductivity	µS/cm at 25˚C	1 to 75,000	1.0	2.0	2.0	20	± 2 or ±10%	± 2 or 5%
pH	Std units	3.3 to 10.2	N/A	NA	NA	5.75, 8.25	≤5.75 or ≥ 8.25 = ±0.07; 5.75-8.25 = ±0.15	≤5.75 or ≥ 8.25 =±0.15; 5.75-8.25 = ±0.05
Ammonia (NH_3)	mg N/L	0 to 17	0.01 marine (0.7 µeq/L) 0.02 freshwater	0.02 (1.4 µeq/L)	Max of 0.1 marine* 0.02 freshwater	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Nitrate-Nitrite (NO_3-NO_2)	mg N/L	0 to 360 (as nitrate)	0.01 marine 0.02 freshwater	0.02	0.05 marine Max of 0.05 freshwater*	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Nitrogen (TN)	mg/L	0.1 to 90	0.01	0.02	Calculated	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Phosphorous (TP)	µg P/L	0 to 22,000 (as TP)	2.0	4.0	10	20.0	± 2 or ±10%	± 2 or ±10%
Dissolved Organic Carbon (DOC)	mg C/L	0.1 to 109	0.1	0.20	0.5	≤ 1 > 1	± 0.10 or ±10%	± 0.10 or ±10%
Turbidity	Nephelometric Turbidity Units (NTU)	0 to 44,000	1.0	2.0	2	20	± 2 or ±10%	± 2 or ±10%
Chloride (Cl)	mg Cl/L	0 to 5,000	0.10 (3 µeq/L)	0.20 (6 µeq/L)	Max of 1*	1	± 0.10 or ±10%	± 0.10 or ±10%
Sulfate (SO_4)	mg SO_4/L	0 to 5,000	0.25 (5.2 µeq/L)	0.50 (10.4 µeq/L)	Max of 1*	2.5	± 0.25 or ±10%	± 0.25 or ±10%
Chlorophyll-*a*	µg/L in extract	0.7 to 11,000	0.5	0.5	0.5**	15	± 1.5 or ±10%	± 1.5 or ±10%

[1] Estimated from samples analyzed for NWCA 2011 and at the EPA Western Ecological Division-Corvallis laboratory between 1999 and 2005

[2] The method detection limit is determined as a one-sided 99% confidence interval from repeated measurements of a low-level standard across several calibration curves.

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

[4] 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.

[5] Accuracy is estimated as the difference between the measured (across batches) 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.

* The national lab contractor shall provide the results of additional development with ion chromatography in attempting to achieve EPA's reporting limits. If EPA determines that the contractor has made a good faith effort, EPA will accept the reporting limits that the contractor has been able to achieve up to a maximum of the value shown in Table 6-4.

** The reporting limit assumes that the field crew provide enough filtered sample so that the lab does not need to adjust the reporting limit.

Table 6-5 Water Chemistry and Chlorophyll-a: Analytical Methods Used in Past NARS Surveys (EPA ORD-Corvallis)

Analyte	Summary of Method[1]	References[2]	WRS SOP[3]
pH (lab)	Automated, using ManSci PC-Titrate w/ Titra-Sip autotitrator and Ross combination pH electrode. Initial pH determination for ANC titration	EPA 150.6 (modified)	WRS 16A.0 (April 2011)
Conductivity	Electrolytic, Man-Tech TitraSip automated analysis OR manual analysis, electrolytic	EPA 120.6	WRS 16A.0 (April 2011) WRS 11A.4 (April 2011)
Nitrate+Nitrite, as N	Ion Chromatography (freshwater samples) OR FIA automated colorimetric (cadmium reduction for brackish or freshwater samples)[4]	EPA 300.6; SW-846 9056A; APHA 4110B EPA 353.2 APHA 4500-NO_3-N-E Lachat 10-107-04-1-C	WRS 36A.0 (April 2011 WRS 40A.5 (May 2011)
Chloride and Sulfate (potential)	Ion Chromatography (freshwater samples)	EPA 300.6; SW-846 9056A; APHA 4110B	WRS 40A.5 (May 2011)
Ammonia, as N	FIA automated colorimetric (salicylate, dichloroisocyanurate)	PEA 350.1, or modification Lachat 10-107-06-3-D	WRS 30A.4 (April 2011)
Total nitrogen (TN)	Persulfate Digestion; FIA Automated Colorimetric Analysis (Cadmium Reduction, sulfanilamide)	EPA353.2 (modified) APHA 4500-N-C (modified) ASTM WK31786 U.S. EPA (1987) Lachat 10-107-04-1-C (modified)	WRS 34A.5 (April 2011)
Total phosphorus (TP)	Persulfate Digestion	EPA 365.1	WRS 34A.5 (April 2011)
Dissolved Organic Carbon (DOC)[5]	UV promoted persulfate oxidation to CO_2 with infrared detection	APHA 5310-C U.S. EPA (1987)	WRS 21A.4 (May 2011)
Turbidity	Nephelometric; Man-Tech TitraSip automated analysis, OR Manual analysis using Hach turbidimeter (high turbidity samples)	APHA 214 A, EPA 180.1 U.S. EPA (1987)	WRS 16A.0 (April 2011) WRS 13A.3 (April 2011)
Chlorophyll-a (CHLA)	Extraction 90% acetone analysis by fluorometry	EPA 445.0 , EPA 446.0	WRS 71A.3 (April 2011)

[1] FIA=Flow injection analysis. AAS=Atomic Absorption Spectrometry

[2] U.S. EPA, 1987. *Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry.* EPA/600/4-87/026. U.S. Environmental Protection Agency, Office of Research and Development, Washington D.C. APHA= American Public Health Association (*Standard Methods*). ASTM=American Society of Testing and Materials.

[3] WRS= Willamette Research Station. References are to laboratory SOP being used at central laboratory. Available upon request from the EPA HQ Laboratory Review Coordinator.

[4] Brackish samples that require use of the FIA method are those above 9000 uS/cm while those below 9000 uS/cm are considered freshwater and can be run using either the IC or FIA method.

[5] For DOC, "dissolved" is defined as that portion passing through a 0.45 µm nominal pore size filter. For other analytes, "dissolved" is defined as that portion passing through a 0.4 µm pore size filter (Nucleopore or equivalent).

6.7 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.

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 (CHLA)	
REPEAT	Numeric	Duplicate	
DATE_COLLECT	Date	Date that the field crew collected the sample	
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 quality control 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	No. Significant Figures	Maximum No. Decimal Places
Temperature	°C	2	1
pH	pH units	3	2
Dissolved Organic Carbon	mg/L	3	1
Conductivity	µS/cm at 25 °C	3	1
Total phosphorus	µg/L	3	0
Total nitrogen	mg/L	3	2
Nitrate-Nitrite	mg/L	3	2
Ammonia	mg/L	3	2
Turbidity	NTU	3	0
Chlorophyll *a*	ug/L	3	2
Chloride and sulfate (Potential)	mg/L	3	1

6.8 Quality Measures

This section describes the quality assurance and quality control 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 quality control 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 give a unique sample identification. Table 6-8 provides a summary of the quality control requirements.

Table 6-8 Water Chemistry and Chlorophyll-a: Quality control 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 A	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; missing label; 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 1.
Store sample appropriately.	All	Check the temperature of the refrigerator per laboratory's standard operating procedures.	Record temperature of sample 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 a maximum temperature of 4° 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. 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. EPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.
Analyze Laboratory/ Reagent Blank	All		Once per day prior to sample analysis	Control limits ≤ MDL	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.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Analyze Filtration Blank	All dissolved analytes	ASTM Type II reagent water processed through filtration unit	Prepare once per week and archive Prepare filter blank for each box of 100 filters, and examine the results before any other filters are used from that box.	Measured concentrations <MDL	Measure archived samples if review of other laboratory blank information suggest source of contamination is sample processing.
Determine LT-MDL Limit for Quality Control Check Sample (QCCS)	All	Prepared so concentration is four to six times the LT-MDL objective	Once per day	Target LT-MDL value (which is calculated as a 99% confidence interval)	Confirm achieved LRL by repeated analysis of LT-MDL QCCS. Evaluate affected samples for possible re-analysis.
Analyze Calibration QCCS	All		Before and after sample analyses	±10% or method criteria	Repeat QCCS analysis. Recalibrate and analyze QCCS. Reanalyze all routine samples (including PE and field replicate samples) analyzed since the last acceptable QCCS measurement.
Analyze Laboratory Duplicate Sample	All		One per batch	Control limits < precision objective	If results are below LRL: Prepare and analyze split from different sample (volume permitting). Review precision of QCCS measurements for batch. Check preparation of split sample. Qualify all samples in batch for possible reanalysis.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Analyze 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.
Analyze Matrix Spike Samples	Only prepared when samples with potential for matrix interferences are encountered		One per batch	Control limits for recovery cannot exceed 100±20%	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).
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.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
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.

*Chapter 2 and Appendix A provides contact information for the EPA HQ NWCA Laboratory Review Coordinator. Laboratories under contract to EPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the Laboratory Review Coordinator.

6.9 Sample and Record Retention

The laboratory shall retain:

1. 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.
2. 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.

6.10 Literature Cited

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, 1987. Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry. EPA/600/4-87/026. U.S. Environmental Protection Agency, Office of Research and Development, Washington D.C.

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, retrieved June 30, 1997 from http://www.epa.gov/microbes/documents/marinmet.pdf.

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", retrieved June 30, 2014 from http://www.epa.gov/microbes/documents/m353_4.pdf.

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., retrieved June 30, 2014 from http://pubs.usgs.gov/of/1999/0193/report.pdf.

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 ALGAL TOXIN (MICROCYSTIN) IMMUNOASSAY PROCEDURE[1]

This chapter describes an immunoassay procedure that measures concentrations of total microcystins in water samples. In applying the procedure, the laboratory uses Abraxis' Microcystins-ADDA Test Kits ("kits", Figure 7-1). Each kit is an enzyme-linked immunosorbent assay (ELISA) for the determination of microcystins and nodularins in water samples. Microcystins refers to the entire group of toxins, all of the different congeners, rather than just one congener. Algae 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.

Figure 7-1 Microcystin: Abraxis microcystin test kit (from James, page 3, 2010)

7.1 Summary of Method

The procedure is an adaption of the instructions provided by Abraxis for determining total microcystins concentrations using its ELISA-ADDA kits.[2] 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

[1] Algal toxin samples collected in NWCA will be processed and analyzed by the USGS Organic Geochemistry Research Laboratory (OGRL) and by State operated laboratories. The SOP used by the USGS OGRL to analyze for the algal toxin microcystin is provided in Appendix F.

[2] Abraxis, "Microcystins-ADDA ELISA (Microtiter Plate): User's Guide R021412." Retrieved on January 14, 2014 from http://www.abraxiskits.com/uploads/products/docfiles/278_Microcystin%20PL%20ADDA%20users%20R120214.pdf.

preparation steps for samples with salinities≥3.5 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.

7.2 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:

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 eyeware, gloves).
3. 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.

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

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

7.3.1 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 ABRAXIS microcystins test procedure, are samples with salinity greater than or equal to 3.5 parts per thousand (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. If the value is:

- < 0.10 µg/L, then the laboratory reports the result as being non-detected ("<0.10 µg/L").
- 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, the laboratory must dilute and reanalyze the sample.

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.

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."

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. Per Section 7.6.4, controls are evaluated in duplicate or triplicate (i.e., three aliquots).

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 quality control requirement in terms of %CV instead of RSD.

Reporting Limit: For undiluted freshwater sample, the reporting limit is 0.15 µg/L. A reporting limit is the point at which the measured value of the analyte can be reported with confidence.

Standard Deviation (S) shows variation from the average

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method's detection limit of 0.1 µg/L. For diluted samples, the sample-specific detection limit will be the product of the method's detection limit of 0.1 µg/L and the dilution factor. Typical values for the dilution factor will be 10 or 100.

Seawater Sample: See definition for brackish and seawater samples.

7.4 General Requirements for Laboratories

7.4.1 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.

7.4.2 Quality assurance and quality control requirements

To demonstrate its expertise in quality assurance and quality control 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 Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NWCA 2016 QAPP Certification Page.

7.4.3 Personnel

Laboratory Technician: This procedure may be used by any laboratory technician who is familiar with the NWCA 2016 QAPP, and this procedure in the NWCA 2016 LOM (which differs from the Abraxis instructions). The laboratory technician also must be familiar with the use of a multichannel pipette and plate readers.

External QC Coordinator is an EPA staff person who is responsible for selecting and managing the "**QC contractor**." To eliminate the appearance of any inherent bias, the QC contractor must be dedicated to QA/QC functions, and thus, must not be a primary laboratory or a field sampling contractor for NWCA. The QC contractor is responsible for complying with instructions from the External QC Coordinator; coordinating and paying for shipments of the performance samples to participating laboratories; comparing immunoassay results from the laboratories; and preparing brief summary reports.

7.4.4 Equipment/Materials

The procedures require the following equipment and information:

- Abraxis 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 Appendix C.
- Distilled or Deionized Water: For diluting samples when necessary.
- ELISA evaluation software
- Glass scintillation, LC, vials (two vials of 2 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)

7.5 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 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.

1. Report receipt of samples in the NARS IM sample tracking system (within 24 clock hours).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
    a. Verify 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. Record the information in Table 7-1 into NARS IM, including the Condition Code for each sample:
        i. *OK*: Sample is in good condition
        ii. *C*: Sample container was cracked
        iii. *L*: Sample container is leaking
        iv. *ML*: Sample label is missing
        v. *NF*: Sample not frozen
    c. If any sample is damaged or missing, contact the EPA HQ Laboratory Review Manager to discuss whether the sample can be analyzed. (See contact information in Table 2-1).

3. Store samples in the freezer until sample preparation begins.
4. Maintain the chain of custody or sample tracking forms with the samples.

Table 7-1 Microcystin: 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**
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		W	Sample is warm (>8 ^{o}C)
		Q	Other quality concerns, not identified above
CONDITION COMMENT	text	Comments about the condition of the sample. If the condition code='W' then provide the temperature	

7.6 Procedure

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

7.6.1 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^{o} 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 other similar alternative are acceptable. One new syringe and filter should be used per sample.

7.6.2 Additional Sample Preparation for Samples with Salinity>3.5 parts per thousand

For any sample with salinity of 3.5 parts per thousand (ppt) or greater (the salinity will be marked on sample vials), the laboratory technician needs to perform the following additional steps provided by Abraxis. [1] For all other samples (i.e. with salinity less than 3.5 ppt), the technician skips this section (i.e., Section 7.6.2) and goes directly to kit preparation as described in Section 7.6.3. 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. [2]. 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.

1 Reformatted from Abraxis, "Microcystins in Brackish Water or Seawater Sample Preparation" Retrieved on January 14, 2014 from http://abraxiskits.com/uploads/products/docfiles/385_MCT-ADDA%20in%20Seawater%20Sample%20Prep%20%20Bulletin%20R041112.pdf. Reproduced with permission. Except for Abraxis' solutions labeled as seawater, EPA has removed references to "brackish" and "seawater" which typically are defined as having different cutpoints than 3.5 ppt for salinity.

2 Additional correspondence between EPA and Abraxis notes that this step leaves the resin in the column.

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 Abraxis Microcystins-ADDA ELISA Kit beginning with the next section (7.6.3).

7.6.3 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 of the required contents:
    - Microtiter plate
    - Standards (6) referenced in this procedure as follows with the associated concentration:
        - S0: 0 µg/L
        - S1: 0.15 µg/L
        - S2: 0.40 µg/L,
        - S3: 1.0 µg/L
        - S4: 2.0 µg/L
        - S5: 5.0 µg/L
    - Kit Control (KC): 0.75 µg/L
    - Antibody solution
    - Anti-Sheep-HRP Conjugate

- Wash Solution 5X Concentrate
- Color Solution
- Stop Solution
- Diluent

3. If any bottles are missing or damaged, discard the kit. This step is important because Abraxis 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.

7.6.4 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 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 7-2) 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 Manager for approval prior to first use. (See Section 2 of the manual 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 7-2. 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	NC	U4	U8	U12	U16	U20	U24	U28	U32	U36
B	S0	S4	NC	U4	U8	U12	U16	U20	U24	U28	U32	U36
C	S1	S5	U1	U5	U9	U13	U17	U21	U25	U29	U33	U37
D	S1	S5	U1	U5	U9	U13	U17	U21	U25	U29	U33	U37
E	S2	KC	U2	U6	U10	U14	U18	U22	U26	U30	U34	U38
F	S2	KC	U2	U6	U10	U14	U18	U22	U26	U30	U34	U38
G	S3	KC	U3	U7	U11	U15	U19	U23	U27	U31	U35	U39
H	S3	NC	U3	U7	U11	U15	U19	U23	U27	U31	U35	U39

Figure 7-2 Microcystin: sample template

Key:
S0-S5 = Standards;
KC = Control supplied with Kit (i.e., Kit Control);
NC = Negative Control;
U = Unknown (sample collected by the field crew).

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:
        iii. $\bar{A}_0 > \bar{A}_1 > \bar{A}_2 > \bar{A}_3 > \bar{A}_4 > \bar{A}_5$
        iv. The average absorbance of the standard S0 less than 0.8 (i.e., $\bar{A}_0 < 0.8$).
        v. 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.
        vi. 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 7.6.5
        ii. The %CV > 15% between the duplicate absorbance values for a sample.
24. Record the results, even if the data failed the quality control 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; U for unknown sample.
    b. **CONC** contains the numeric concentration value. Two special cases:
        i. Non-detected concentrations: If the sample is non-detected, then provide the sample-specific detection limit which is 0.1 µg/L if the sample is undiluted. See Section 7.3.1 for calculating the sample-specific detection limit for a diluted sample.

ii. If the result shows that it is "HI," 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. **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 Microcystin: 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
			W	Sample is warm (>8 ^{o}C)
			Q	Other quality concerns, not identified above
	CONDITION COMMENT	text	Comments about the condition of the sample. If the condition code='W' then provide the temperature	

STAGE	FIELD	FORMAT	DESCRIPTION	
ANALYSIS	TECHNICIAN	text	Name or initials of technician performing the procedure	
	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
			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)	
	SALINITY	numeric	If the sample vial has the salinity marked on the vial, record the value in units of parts per thousand. Otherwise, leave blank.	
	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 if the sample hasn't been diluted.	
	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. Substitute 0.15 µg/L for any result recorded as <0.15 µg/L	
	DATA FLAG (if appropriate)	text	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).	
			Flag	**Definition**
			ND	Concentration below detection. Unless the sample was diluted, the concentration will be 0.1 µg/L
			HI	Result indicated that a high concentration (i.e., outside calibration range)
			J	Concentration above detection but below reporting limit. Without dilution, these values are between 0.1 and 0.15 µg/L
	QUAL_FLAG	QCF/Q	QCF	QC failure
			Q	Other quality concerns, not identified above
	COMMENTS	text	Explanation for data flag(s) (if needed) or other comments.	

7.6.5 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 Abraxis' 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 Abraxis' 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.

7.7 Quality Measures

This section describes the quality assurance and quality control measures used to ensure that the data will meet NWCA requirements.

7.7.1 Assistance Visits

Assistance visits 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. If EPA decides to conduct an assistance visit, a qualified EPA scientist or contractor will administer a checklist based upon the steps described in this chapter.

7.7.2 QC Samples

During the course of the survey, the External QC Coordinator will instruct the QC contractor to provide one or two identical sets of QC samples to all participating laboratories. Each set will contain up to five QC samples. As determined by the External QC Coordinator, the QC samples may be synthetic; aliquots of additional samples collected at NRSA reference sites; or reference samples obtained from an organization such as the National Institute of Standards. Each laboratory will run the QC samples following the same procedures used for the other samples. The QC contractor 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, 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.

7.7.3 Summary of QA/QC Requirements

Table 7-3 provides a summary of the quality control requirements for procedures described in Section 7.6.

Table 7-3 Microcystin: quality control – sample analysis

Quality Control 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.
Calibration	All of the following must be met: o Standard curve must have a correlation coefficient of ≥0.99; o Average absorbance value, $\bar{A}_0$, for S0 must be >0.80; and o 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 calibtration provides acceptable results.
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: o All concentration values must be < 0.15 µg/L (i.e., the reporting limit); and o One or more concentration results must be nondetectable (i.e., <0.10 µg/L)	

Quality Control Activity	Description and Requirements	Corrective Action
Sample Evaluations	All samples are run in duplicate. Each duplicate pair must have %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. 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%).
Results Within Calibration Range	All samples are run in duplicate. If both of the values are less than the upper calibration range (i.e., 5.0 µg/L for undiluted samples), then the requirement is met.	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.**
External Quality Control 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.

7.8 Sample and Record Retention

The laboratory shall retain:

1. 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.

2. 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.

7.9 References

Abraxis, "Microcystins-ADDA ELISA (Microtiter Plate)," Product 520011, R021412, Undated. Retrieved January 2014 from http://www.abraxiskits.com/uploads/products/docfiles/278_Microcystin%20PL%20ADDA%20users%20R120214.pdf.

Abraxis, "Microcystin-ADDA ELISA Kit, Detailed Procedure," Undated. Retrieved January 2014 from http://www.abraxiskits.com/uploads/products/docfiles/253_PN520011FLOW.pdf.

James, 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 March 2013 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's Office of Research and Development will process and analyze these samples under a cooperative agreement with Michigan State University and Kenyon College.

APPENDIX A: CONTACT INFORMATION

Title	Name	Contact Information
EPA HQ NWCA Project Manager	Gregg Serenbetz, OW	serenbetz.gregg@epa.gov 202-566-1253
EPA HQ NWCA Alternate Project Manager	Chris Faulkner, OW	Faulkner.chris@epa.gov 202-566-1185
EPA HQ NARS QA Lead	Sarah Lehmann, OW	lehmann.sarah@epa.gov 202-566-1379
EPA HQ Logistics Lead	Colleen Mason, OW	Mason.colleen@epa.gov 202-343-9641
EPA HQ NWCA Laboratory Review Coordinator	Kendra Forde, OW	kendra.forde@epa.gov 202-564-0417
Information Management Center Coordinator	Marlys Cappaert, SRA International Inc.	cappaert.marlys@epa.gov 541-754-4467

APPENDIX B: LABORATORY REMOTE EVALUATION FORMS

Contents

NWCA 2016 Document Request Form – Chemistry Laboratories

The U.S. EPA, states and other partners are planning the second National Wetland Condition Assessment (NWCA) for 2016. The survey uses a probability-based sampling design to represent the condition of wetlands across the Continental United States by sampling at approximately 1200 sites. Consistent sampling and analytical procedures ensure that EPA can compare the results across the country and over time.

As part of the 2016 NWCA, the Quality Assurance Team has been requested to conduct a technical assessment to verify quality control practices in your laboratory and its ability to perform chemistry 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 2016 NWCA.

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 an on-site visit. All labs will need to complete the following forms:

All laboratories will be required to complete the following forms and check the specific parameter in which your laboratory will be conducting an analysis for the 2016 NWCA:

- ☐ Water Chemistry and Chlorophyll-*a* (all of the analytes identified in the LOM and QAPP)
- ☐ Microcystin

If your lab has been previously approved within the last 5 years for the water chemistry indicator:

- ☐ A *signature* on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for chemistry labs conducting analyses for the 2016 NWCA.
- ☐ A signature on the Quality Assurance Project Plan (QAPP) and the Laboratory Operations Manual (LOM) Signature Form indicates that you will follow both the QAPP and the LOM.

If you have not been approved within the last 5 years through the laboratory verification process for the water chemistry indicator, in order for us to determine your ability to participate as a laboratory in the NWCA, we are requesting that you submit the following documents (if available) for review:

- ☐ Documentation of a successful *quality assurance audit* from a prior National Aquatic Resource Survey (NARS) that occurred within the last 5 years.
- ☐ Documentation showing participation in a previous NARS for Water Chemistry for the same parameters/methods.

Additionally, we request that all labs provide the following information in support of your capabilities, (these materials are required if neither of the two items above are provided):

- ☐ 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 2016 NWCA LOM).
- ☐ Documentation attesting to experience running all analytes for the 2016 NWCA, including Chlorophyll *a*.

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 2016 National Wetland Condition Assessment (NWCA).

This applies to the _____ chemistry indicator(s).

1.) Use procedures identified in the 2016 NWCA Laboratory Operations Manual (or equivalent). If using equivalent procedures, please provide the procedures and obtain approval from EPA.
2.) Read and abide by the 2016 NWCA Quality Assurance Project Plan (QAPP) and related Standard Operating Procedures (SOPs).
3.) Have an organized IT tracking system in place for recording sample tracking and analysis data.
4.) Provide Quality Control (QC) data for internal QC check, on a quarterly basis.
5.) Provide data using the template provided on the NARS Sharefile.
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 May 1, 2017 or as otherwise negotiated with EPA.
7.) Participate in a laboratory technical assessment or audit if requested by EPA NWCA staff (this may be a conference call or on-site audit).
8.) 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 2016: 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 2016 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 2016 NWCA 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 with Chris Faulkner (EPA HQ NWCA Project Manager-Alternate, Faulkner.Chris@epa.gov).

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 five known plant voucher samples (randomly selected from species identified by the Vegetation Team) for quality assurance (NWCA 2016 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 2016 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 vegetation 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 chapter 4 of the LOM, to ensure that all QA vouchers are correctly verified.

VEGETATION LABORATORY QUALITY EVALUATIONS
NWCA 2016 VEGETATION LABORATORY ASSISTANCE CHECKLIST

Lab ID: _____ Individuals Performing IDs: _____

Individuals on Conference Call: _____

Vegetation Lab Evaluator: _____

Date: _____

Instructions:

1. All vegetation laboratories should be adhering to methods and QA requirements described in the NWCA 2016 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 fill in 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			
Does the herbarium have the following supplies and equipment?			
• Plant dryer	**Y**	**N**	**N/A**
• Dissecting microscope	**Y**	**N**	**N/A**
• Storage cabinet or sealable plastic boxes for storing dried plant samples prior to identification	**Y**	**N**	**N/A**
• Regional floras and plant lists Indicate all floras used by lab:	**Y**	**N**	**N/A**
• Access to USDA PLANTS taxonomic standard (http://plants.usda.gov/java/)	**Y**	**N**	**N/A**
• Plant sample folders	**Y**	**N**	**N/A**

• OPTIONAL: Freezer for freeze treating plant specimens to kill pests. If "N", indicate lab method for killing pests on specimens:	**Y**	**N**	**N/A**
• OPTIONAL: Mounting materials (herbarium sheets, mounting glue, forceps, weights for holding samples with wet glue to the herb Receiving Voucher Samples)	**Y**	**N**	**N/A**
Notes:			
Processing and Managing Plant Samples			
Plant samples may arrive at the Herbarium as: 1) dried, pressed samples, or 2) pressed but still wet plant material enclosed in a plant press.			
Drying Samples:			
• 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?	**Y**	**N**	**N/A**
• 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?	**Y**	**N**	**N/A**
• If a plant dryer is not available does the lab place the presses in a warm dry place 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?	**Y**	**N**	**N/A**
• 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?	**Y**	**N**	**N/A**
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.*			
• Does the lab have standard treatment procedures for pests and are those procedures being implemented for the NWCA specimens?	**Y**	**N**	**N/A**
• After the samples are pressed and dried, does the lab treat samples for detritivores, molds, and pests?	**Y**	**N**	**N/A**

• 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?	**Y**	**N**	**N/A**
Storing Samples:			
• Are plant samples being stored in herbarium cabinets or sealable plastic container when not in use?	**Y**	**N**	**N/A**
• Does the lab ensure that samples are not left out in the herbarium room overnight? If samples are found that have been left out overnight or if a cabinet/plastic container has been left open, does the lab decontaminate all samples again?	**Y**	**N**	**N/A**
Notes:			

Tracking Specimens – Tracking Form T-2 and T-3, 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-2: NWCA 2016 Unknown Plant Sample Tracking and T-3: NWCA 2016 QA Plant Sample Tracking). It is important that every specimen sent to and received by the lab is tracked following the protocols described in the appropriate section of the LOM Vegetation Chapter.*			
• Does the lab review all the Tracking Forms to ensure that all samples listed are received by the lab?	**Y**	**N**	**N/A**
• If a sample listed on the tracking form is not part of the shipment received, does the lab **contact the Information Management Coordinator (541-754-4663) as soon as possible?**	**Y**	**N**	**N/A**
Plant Specimen Label: *Every sample will arrive at the Herbarium with a Plant Specimen Label. This label includes diagnostic information for known and unknown species collected.*			
• Does the lab review the information provided on the Plant Specimen Label included with the sample? o Plant Sample ID Number: NWCA Site Number-Plant collection number o Sampling Date o Visit Number o County and State of Site o Scientific name for QA Voucher Specimen o Pseudonym for Unknown Species o Collector(s) name(s) o Abundance of Plant o Habitat o Growth Habit	**Y**	**N**	**N/A**

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 Plants (http://plants.usda.gov/).			
• Is the Lab reconciling names for all species that they identify to the standard found in USDA Plants?	**Y**	**N**	**N/A**
• Is the lab a Heritage program or coordinating with a Heritage program or university herbarium?	**Y**	**N**	**N/A**
• Is the lab using a reference herbarium and is it the state's reference herbarium?	**Y**	**N**	**N/A**
• How many unknown plant samples does the lab identify in a year?	# of samples: _____		
Notes:			
Mounting and Storing Herbarium Sheets			
Once the samples are dried, pressed, and identified, they are to be stored at the Herbarium for at least five years.			
• Does the lab have the storage capabilities and facilities to properly store dried, pressed, and identified samples for at least five years?	**Y**	**N**	**N/A**
• Will vouchers be kept in sealable plastic containers in a cool dry climate and will they be accessible to the EPA?	**Y**	**N**	**N/A**
• Will the lab incorporate the NWCA vouchers into their permanent collections?	**Y**	**N**	**N/A**
• Will vouchers from the national survey be mounted on herbarium sheets and labeled to indicate that they were collected as part of the NWCA?	**Y**	**N**	**N/A**
Notes:			

Sending Resultant Data Forms			
Data must be reported to EPA electronically using the 2016_NWCA Plant ID_Lab Spreadsheets.xlsx data template. The template includes separate spreadsheets for recording names of identified unknowns and reconciling to PLANTS nomenclature and for recording re-identification and verification of QA voucher specimens.			
• The lab is aware of these reporting requirements and is sending in the resultant data per the instructions in *2016_NWCA Plant ID_Lab Spreadsheets.xlsx* data templates.	**Y**	**N**	**N/A**
• The species identifications are regularly entered into the appropriate spreadsheets, and these forms are transmitted at appropriate intervals to the EPA Project Management Team.	**Y**	**N**	**N/A**
Notes:			

Quality Assurance/Quality Control			
A subset of plant samples collected as unknowns 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-3) needs to be completed and sent with the specimens. Additionally, five randomly selected species (from each AA) of known identity will be reassessed by an independent botanist/taxonomist. (QAPP section 5.1.5).			
• For QA, if the field botanist/ecologist is acting as the laboratory/herbarium, does the lab ensure that another qualified botanist, or a state or EPA identified laboratory/herbarium is used for QA?	**Y**	**N**	**N/A**
• 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")?	**Y**	**N**	**N/A**
• Does the lab record all identifications in the 2016 NWCA Plant ID Lab Spreadsheet for each sample, and email to the EPA Project Management Team?	**Y**	**N**	**N/A**
Notes:			

Laboratory Signature Form – Vegetation Laboratory/Herbarium

I certify that the laboratory/herbarium, located in , will abide by the following standards in performing the following data analysis and reporting for the 2016 National Wetland Condition Assessment (NWCA).

This applies to the _____ vegetation indicator.

1.) Use procedures identified in the 2016 NWCA Laboratory Operations Manual (or equivalent). If using equivalent procedures, please provide the procedures and obtain approval from EPA.

2.) Read and abide by the 2016 NWCA Quality Assurance Project Plan (QAPP) and any related Standard Operating Procedures (SOPs).

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

4.) Notify EPA 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 Sharefile.

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 May 1, 2017 or as otherwise negotiated with EPA.

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

Signature _____ Date _____

APPENDIX C: DATA REPORTING TEMPLATES

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

2016_NWCA Plant ID_Lab Spreadsheets.xlsx
2016_NWCA Water Chem-CHLA_Lab Spreadsheet.xlsx
2016_NWCA Microcystin Lab Spreadsheet.xlsx

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

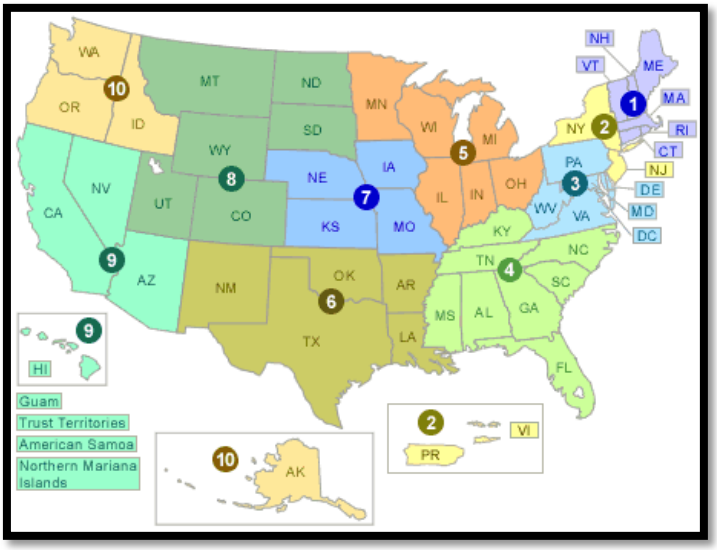
Recording Citations for Floristic Resources

The nomenclatural standard for the NWCA is the PLANTS Database (USDA, NRCS 2016, http://plants.usda.gov/). Ideally, plant species names based on PLANTS nomenclature would be recorded during data collection. However, for plant identification in the field or lab it is often necessary to use local or regional floras and field guides, which may represent different nomenclature.

Partial List of Regional, State, and Local Floras and Field Guides

A list of floras and field guides is provided below. This table does not represent a complete listing of floras and field guides available for the conterminous US, but is largely based on the list of floras and field guides that the Botanist/Ecologists on the Field Crews selected and reported using in the NWCA 2011. In addition, several other floristic resources are included. Additonal printed and online floras, not included in this list, are likely to also be useful (particularly those recently published).

Resources are alphabetized by author. The states in which they were used in the NWCA are listed. Use of a floristic reference in a particular state does not necessarily mean that its utility is limited to that state. Often floras may have regional applicability. To help consider regional utility, the EPA Regions that include the states where the floras and field guides were used in 2011 are also listed. However, it is important to note that EPA Regions do not necessarliy represent ecological boundaries and may exceed the area to which a flora applies, or conversely may not include adjacent area that may be covered by a particular flora.



Map of EPA Regions

Potentially Useful Floras and Field Guides	EPA Region(s) Where ***Potentially*** Applicable	Used by States in NWCA 2011
Allen, C. M., D. A. Newman, and H. W inters. 2004. *Grasses of Louisiana*. 3rd ed. Allen's Native Adventures, Pitkin, LA	Region 6	LA, MS
Allen, Charles M., Dawn Allen Newman, and Harry H. Winters. 2002. *Trees, shrubs, and woody vines of Louisiana*. Allen's Native Ventures, LLC, Pitkin, LA.	Region 6	LA, MS
Allred, Kelly. 2005. *A Field Guide to the Grasses of New Mexico*. Third Edition. New Mexico State University. Las Cruces, New Mexico.	Region 6	NM
Allred, K.W. and R.D. Ivey. DRAFT. *Flora Neomexicana. Volume III: An illustrated identification guide to the vascular plants of New Mexico*. New Mexico State University.	Region 6	NM
Allred, K.W. and R.D. Ivey. 2010. *Flora Neomexicana* (DRAFT). Published by the authors.	Region 9	AZ
Anderton, L.K., and M.E. Barkworth. 2009. *Grasses of the Intermountain Region*. Intermountain Herbarium, Utah State University, Logan, UT 84322	Region 9	NV
Barkely, T.M. 2006. *Senecio*. In: *Flora of North America* Editorial Committee, eds. 1993+.Flora of North America North of Mexico. 16+ vols. New York and Oxford. Vol. 20: Magnoliophyta: Asteridae: Asteraceae, part 2. Accessed via www.efloras.org. Summer 2011.	All	ID
Barkley, T.M., L. Brouillet, J.L. Strother. 2006. *Asteraceae. In: Flora of North America*. Editorial Committee, eds. 1993*+. Flora of North America North of Mexico*. 16+ vols. New York and Oxford. Vols. 19, 20, and 21: Magnoilophyta: Asteridae: Asteraceae, part 1, part 2, part 3. Accessed via www.efloras.org. Summer 2011.	All	ID
Barneby, R.C. 1989. *Intermountain Flora: Vascular Plants of the Intermountain West, U.S.A. Volume 3, Part B: Fabales*. New York Botanical Garden Press, New York, 292 pp.	Regions 8, 9, 10	ID
Beidleman, L.H., R.G. Beidleman, and B.E. Willard. 2000. *Plants of Rocky Mountain National Park*. Falcon Press, Helena, Montana, 266 pp.	Region 9	AZ
Beidleman, L.H. and E. Kozloff. 2003. *Plants of the San Francisco Bay Region*. University of California Press, Berkeley, California, 514 pp.	Region 9	CA
Bell, C.R. and B.J. Taylor. 1982. *Florida wild flowers and roadside plants*. Laurel Hill Press, Chapel Hill, North Carolina, 308 pp.	Region 4	FL
Belliston, N.D., J. Merritt, R. Whitesides, and S.A. Dewey. 2004. *Noxious Weed Guide for Utah*. Utah State University Extension. 50 pp.	Region 8	UT
Black, M.R. and E.J. Judziewiez. 2009. *Wildflowers of Wisconsin and the Great Lakes Region: A Comprehensive Field Guide*. Second Edition. The University of Wisconsin Press. Madison, Wisconsin. 277 pp.	Region 5	WI
Braun, L.E. 1967. *The Vascular Flora of Ohio. Part 1: The Monocotyledoneae: Cat-tails to Orchids*. The Ohio State University Press. Columbus, Ohio, 464 pp.	Region 5	OH
Brown, C. L., and K. Kirkman. 1990. *Trees of Georgia and Adjacent States.* Timber Press. Portland, Oregon.	Region 4	FL

Potentially Useful Floras and Field Guides	**EPA Region(s) Where *Potentially* Applicable**	**Used by States in NWCA 2011**
Brunsfeld, S. J., and F.D. Johnson. 1985. *Field guide to the willows of east-central Idaho*. Bulletin Number 39, Forest, Wildlife, and Range Experiment Station, University of Idaho, Moscow, Idaho.	Region 10	ID, MT
Center for Aquatic and Invasive Plants, University of Florida, IFAS website address: http://plants.ifas.ufl.edu/		FL
Chadde, S.W. 2002. *A Great Lakes Wetland Flora*. Second Edition. Pocketflora Press. Laurium, Michigan, 648pp.	Region 5	IL, IN, MI, OH, WI
Clements, Steven. 1992. *Chenopodiaceae and Amaranthacae of New York State*. Bulletin No. 485. New York State Museum. Albany, New York.	Region 2	NJ
Clewell, A.F. 1985. *Guide to the Vascular Plants of the Florida Panhandle*. Florida State University Press.	Region 4	FL
Colvin, et al. 2004. *Weeds of Southern Turfgrass*. University of Florida, IFAS Extension.	Region 4	FL
Cooke, S.S. 1997. *A Field Guide to the Common Wetland Plants of Western Washington & Northwestern Oregon*. Seattle Audubon Society 403 pp.	Region 10	WA
Cooperrider, T. S. 1995. *The Dicotyledonae of Ohio Part 2: Linaceae through Campanulaceae*. The Ohio State University Press, Columbus, Ohio.	Regions 3 and 5	OH
Cope, E.A. 2001. *Muenscher's Keys to Woody Plants: An Expanded Guide to Native and Cultivated Species*. Cornell University Press. Ithaca, New York, 321 pp.	Region 1	NH
Cronquist A. and others. 1977+. *Intermountain Flora*. Columbia University Press or New York Botanical Garden Press, New York.	Regions 8, 9, 10	AZ, NV, UT
Cronquist, A. 1994. *Intermoutain Flora: Vascular Plants of the Intermountain West, U.S.A. Volume 5: Asterales*. The New York Botanical Garden Press, New York, 506 pp.	Regions 8, 9, 10	ID
Cronquist, A. N.H. Holmgren, and P. K. Holmgren. 1997. *Intermoutain Flora: Vascular Plants of the Intermountain West, U.S.A. Volume 3, Part A: Subclass Rosidae (except Fabales)*. New York Botanical Garden Press, New York, 456 pp.	Regions 8, 9, 10	ID
Crow, G.E., C.B. Hellquist, and N.C. Fasset. 2006. *Aquatic and Wetland plants of Northeastern North America. Vol. 1. Pteridophytes, Gymnosperms, and Angiosperms Dicotyledons*. The University of Wisconsin Press, 448 pp.	Regions 1, 2, 3, 6	MN
Crow, G.E. and C.B. Hellquist. 2000. *Aquatic and Wetland plants of Northeastern North America. Vol. 2. Angiosperms: Monocotyledons.* The University of Wisconsin Press, 464 pp.	Regions 1, 2, and 3	NH
Culver, D.R. and J.M. Lemly. 2013. Field Guide to Colorado's Wetland Plants: Identification, Ecology, and Conservation. Colorado Natural Heritage Program, Colorado State University, Fort Collins, CO		CO
DiTomaso, J.M. and E.A. Healy. 2006. *Weeds of California and Other Western State*s. University of California Division of Agriculture and Natural Resources, Publication 3488.	Region 9	CA
Dorn, R.D. and J.L Dorn. 1984. *Vascular Plants of Montana.* Mountain West Publishing, Cheyenne, Wyoming, 276 pp.	Region 8	MT, WY

Potentially Useful Floras and Field Guides	EPA Region(s) Where ***Potentially*** Applicable	Used by States in NWCA 2011
Dorn, R.D. 2001. Vascular Plants of Wyoming. Third Edition. Mountain West Publishing. Cheyenne, Wyoming.	Region 8	UT, WY
Duncan, W. H., and M. B. Duncan. 1988. *Trees of the Southeastern United States*. University of Georgia Press. Athens, Georgia.	Region 4	FL
Duncan, W.H. and L.E. Foote. 1999. *Wildflowers of the southeastern United States.* University of Georgia Press, Athens, Georgia.	Region 4	GA
Eilers, L.J. and D.M. Roosa. 1994. *The Vascular Plants of Iowa*. University of Iowa Press, Iowa City, Iowa, 319 pp.	Region 7	IA
Farnsworth, A., B. Cobb, and C. Lowe. 2005. *Peterson Field Guide to Ferns*, Second Edition: Northeastern and Central North America. Houghton Mifflin Harcourt. 440 pp.	Region 5	WI
Fassett, N. C. 1957. *A Manual of Aquatic Plants*. Madison: The University of Wisconsin Press. Madison, Wisconsin.		NE, PA
Fleenor, S.B, and S.W. Tabor. 2009. *Plants of Central Texas Wetlands*. Texas Tech University Press. Lubbock, Texas, 275 pp.	Region 6	TX
Flora of North America Editorial Committee, eds. 1993+. *Flora of North America North of Mexico*. 16+ vols. New York and Oxford.	All	AZ, MA, MI, MN, NC, NH, NM, NV, RI, SD, WI, WV
Flora of North America Editorial Committee, eds.1993+. *Flora of North America North of Mexico. Vol. 3: Magnoliidae and Hamamelidae*. New York and Oxford.	All	ID
Flora of North America. Editorial Committee, eds. 1993+. *Flora of North America North of Mexico*. 16+ vols. New York and Oxford. Brooks, R.E. and S.E. Clemants. 2000. *Juncaeae In: Vol. 22: Magnoliophyta: Alismatidae, Arecidae, Commelinidae (in part), and Zingiberidae.* Accessed via www.efloras.org. Summer 2011.	All	ID
Flora of North America Editorial Committee, eds. 1993+.Flora of North America North of Mexico.16+ vols. New York and Oxford. Wolf, S.J. 2006 *Arnica. In: Vol. 21: Mgnoiophyta: Asteridae: Asteraceae , part 3*. Accessed via www.efloras.org. Summer 2011.	All	ID
Flora of North America. Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 16+ vols. New York and Oxford. Brouillet, L., J.C. Semple, G.A. Allen, K.L. Chambers, S.D. Sundberg. 2006. *Symphyotrichum. In: Vol. 20: Mgnoiophyta: Asteridae: Asteraceae, part 2*. Accessed via www.efloras.org. Summer 2011.	All	ID
Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 16+ vols. New York and Oxford. *Morin, N.R. 2009. Ribes. In: Vol. 8: Magnoliophyta: Paeoniaceae to Ericaceae*. Accessed via www.efloras.org. Summer 2011.	All	ID
Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 16+ vols. New York and Oxford. Romero-González, G.A., G.C. Fernádez-Concha, R.L. Dressler, L.K. Magrath, and G.W. Argus. 2002. *Orchidaceae. In: Vol. 26: Magnoliophyta: Liliidae: Liliales and Orchdiales.* Accessed via www.efloras.org. Summer 2011.	All	ID

Potentially Useful Floras and Field Guides	EPA Region(s) Where ***Potentially*** Applicable	Used by States in NWCA 2011
Flora of North America Editorial Committee, eds. 1993+.*Flora of North America North of Mexico*. 16+ vols. New York and Oxford. Landolt, E. 2000. *Lemnaceae. In Vol. 22: Magnoliophyta: Alismatidae, Arecidae, Commelinidae (in part), and Zingiberidae.*	All	MA
Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 16+ vols. New York and Oxford. Wiersema, J.H. and B. Hellquist. 1997. *Nymphaceae. In: Vol. 3: Magnoliophyta: Magnoliidae and Hmamelidae.*	All	MA
Flora of North America Editorial Committee, eds. 1993+.*Flora of North America North of Mexico*. 16+ vols. New York and Oxford. *Vol. 7: Magnoliophyta: Salicaceae to Brassicaceae.*	All	MI, MN
Flora of North America Editorial Committee, eds. 1993+.*Flora of North America North of Mexico*. 16+ vols. New York and Oxford. *Vol. 22: Magnoliophyta: Alismatidae, Arecidae, Commelinidae (in part), and Zingiberidae, 2000*.	All	WV
Flora of North America Editorial Committee, eds. 1993+. *Flora of North America North of Mexico.* 16+ vols. New York and Oxford*. Vol. 23: Magnoliophyta: Commelinidae (in part): Cyperaceae. 2002*.	All	WV
Flora of North America Editorial Committee, eds. 1993+. *Flora of North America North of Mexico.* 16+ vols. New York and Oxford. *Vol. 24: Magnoliophyta: Commelinidae (in part): Poaceae (part 1), 2007*.	All	WV
Flora of North America Editorial Committee, eds. 1993*+. Flora of North America North of Mexico*. 16+ vols. New York and Oxford. *Vol. 25: Magnoliophyta: Commelinidae (in part): Poaceae (part 2), 2003.*	All	WV
Foote, L.E. and S.B. Jones, Jr. 2005. *Native shrubs and woody vines of the south-east.* Timber Press, Portland, Oregon.	All	GA
Gleason and Cronquist. 1963. *Manual of the Vascular Plants of Northeastern United States and Adjacent Canada*. Van Nostrand, Princeton NJ. 910 pp.	Regions 1, 2, 3, 4 and 5	MD, MI, MN, ND?, PA, WI
Gleason, Henry A. and Arthur Cronquist. 1991*. Manual of Vascular Plants of the NE U.S. and Adjacent Canada*. Second Edition. New York Botanical Garden. Bronx, NY.	Regions 1, 2, 3, 4 and 5	CT, IL, IN, GA? KY?, MA, ME, NH, NY, OH, PA, RI, TN, VA, WI, WV
Godfrey, R.K. 1988. *Trees, shrubs, and Woody vines of Northern Florida and adjacent Georgia and Alabama*. University of Georgia Press, Athens, Georgia.	Region 4	FL NC, VA,
Godfrey, R. K. and J. W. Wooten. 1981. *Aquatic and wetland plants of the southeastern United States: dicotyledons*. University of Georgia Press, Athens. 933 pp	Regions 4 and 6	AL, AR, GA, LA, MS, NC, TX, VA
Godfrey, R. K. and J. W. Wooten. 1979. *Aquatic and wetland plants of the southeastern United States: monocotyledon*s. University of Georgia Press, Athens. 712 pp.	Regions 4 and 6	AL, AR, FL, GA, LA, MS, NC, SC, TX, VA
Great Plains Flora Association. 1977. *Atlas of the Flora of the Great Plains*. Iowa State Press. Ames, IA.	Regions 7 and 8	KS, ND, SD
Great Plains Floras Association. 1986. *The Flora of the Great Plains*. Coordinator, R.L. McGregor. Editor, T.M. Barkely. University Press of Kansas. Lawrence, Kansas, 1402 pp.	Regions 7 and 8	IA, KS, MO, ND, NE, OK, SD

Potentially Useful Floras and Field Guides	EPA Region(s) Where ***Potentially*** Applicable	Used by States in NWCA 2011
Haines, A. and T.F. Vining. 1998. *Flora of Maine*. V.F. Thomas Company, Bar Harbor, Maine. 847 pp.	Region 1	ME
Haines. 2011. *Flora Novae Angliae*. New England Wildflower Society. (Due for publication in September 2011).	Region 1	ME
Hatch, S.L., Schuster, J.L., and D.L. Drawe, 2003. *Grasses of the Texas Gulf Prairies And Marshes*, Texas A&M University Press, College Station, Texas. 355 pp.	Region 6	TX
Hickman, James C., Editor. 1993. *The Jepson Manual of Higher Plants of California*. University of California Press, Berkeley, California. Third printing with corrections 1996.	Region 9	CA
Hipp, A.L. 2008 *Field Guide to Wisconsin Sedges: An Introduction to the Genus Carex (Cyperaceae*). The University of Wisconsin Press. 280 pp.	Region 5	WI
Hitchcock, A.S. 1971. *Manual of the grasses of the United States. Volume 1*. Dover Publications, New York.	All	ID
Hitchcock, C.L. and A. Cronquist. 1973. *Flora of the Pacific Northwest: An Illustrated Manual*. University of Washington Press, Seattle, Washington, 730 pp.	Regions 8 and 10	ID, MT, OR,WA
Hitchcock, C.L., A. Cronquist, and M. Ownbey. 1969. *Vascular Plants of the Pacific Northwest. Part 1: Vascular Cryptogams, Gymnosperms, and Monocotyledons*. University of Washington Press, Seattle, Washington, 914 pp.	Region 10	ID
Hitchcock, C.L., A. Cronquist, M. Ownbey, and J.W. Thompson. 1971. *Vascular Plants of the Pacific Northwest. Part 3: Saxifragaceae to Ericaceae.* University of Washington Press, Seattle, Washington, 614 pp.	Region 10	ID
Hitchcock, C.L., A. Cronquist, M. Ownbey, and J.W. Thompson. 1959. *Vascular Plants of the Pacific Northwest. Part 4: Ericaceae to Campanulaceae.* University of Washington Press, Seattle, Washington, 510 pp.	Region 10	ID
Hitchcock, C.L., A. Cronquist, M. Ownbey, and J.W. Thompson. 1955. *Vascular Plants of the Pacific Northwest. Part 5: Compositae.* University of Washington Press, Seattle, Washington, 343 pp.	Region 10	ID
Holmgren, Noel H. et al. 1998. *Illustrated Companion to Gleason and Cronquist's Manual*. NY Botanical Garden. Bronx, NY.	Regions 1, 2, 3, and 5	MI, NH, OH, PA
Hurd, E.G., N.L. Shaw, J. Matrogiuseppe, L.C. Smithman, and Sherel Goodrich. 1998. *Field Guide to Intermountain Sedges. General Technical Report. RMRS-GTR-10*. U.S. Department of Agriculture, U.S. Forest Service, Rocky Mountain Research Station, 282 pp.	Regions 8, 9, and 10	CA, ID, MT, NV, UT
Hurd, E.G., S. Goodrich, and N.L. Shaw. 1997 - Revised. *Field guide to Intermountain Rushes.* General Technical Report INT-306. U.S. Department of Agriculture, Forest Service, Intermountain Research Station. Ogden, Utah.	Regions 8, 9, and 10	MT
Ivey, R.D. 2003. *Flowering Plants of New Mexico*. 4th ed. Published by the author.	Region 6 and 8	NM
The Jepson Online Interchange: California Floristics. University of California, Berkley. http://ucjeps.berkeley.edu/interchange/. Accessed 2011	Region 9	CA

Potentially Useful Floras and Field Guides	EPA Region(s) Where ***Potentially*** Applicable	Used by States in NWCA 2011
Jones, R.L. 2005. *Plant Life of Kentucky, An Illustrated Guide to the Vascular Flora*. University Press of Kentucky. Lexington, Kentucky.	Region 4	KY
Kartesz, J. T. 1988. *A Flora of Nevada*. Doctoral dissertation, University of Nevada, Reno. ~vii + 1729 pgs	Region 9	NV
Kershner, B., C. Tufts, G. Nelson, D. Mathews, R. Spellenberg, and T. Purinton. 2008. *National Wildlife Federation Field Guide to Trees of North America*, Sterling Press. 528 pages	All	MS, NC, VA
Kershner, Mathews, Nelson, and Spellenberg. 2008. *National Wildlife Federation Field Guide to Trees of North America*, Chanticleer Press, Inc. p. 229	All	Al, AR, FL, GA, LA
Larson, Gary E. 1993. *Aquatic and wetland vascular plants of the northern Great Plains*. Gen. Tech. Rep. RM-238. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. Fort Collins, Colorado, 681pp.	Region 8	ND, SD
Lavin, M. and C. Seibert. 2011. *Grasses of Montana*. Montana State Univestiy Herbarium. http://www.montana.edu/mlavin/herb/mtgrass.pdf	Region 8	MT
Lazarine, P. 1981. *Common Wetland Plants of Southeast Texas*. U.S. Army Corps of Engineers. Galveston District. Galveston, Texas. 154 pp.	Region 6	TX
Lehman, R.L., R. O'brien and T. White. 2005. *Plants of The Texas Coastal Bend*. Texas A and M University Press, College Station, Texas, 352 Pp.	Region 6	TX
Leppig, G. and A.J. Pickart (compiled by). 2005 *A Photographic Guide to Plants of Humboldt Bay Dunes and Wetland*s. Sponsored by U.S. Fish and Wildlife Service, California Department of Fish and Game, Friends of the Dunes, and National Fish and Wildlife Foundation. http://www.fws.gov/HUMBOLDTBAY/PLANTGUIDE/. Accessed 2011.	Region 9	CA
Lesica, P. and P. Husby. 2006. *Field Guide to Montana's Wetland Vascular Plants*: A non-technical key to the genera with keys to the species of sedges and rushes. 2nd printing. Montana Wetlands Trust. 96 pp.	Region 8	MT
Lesica. 2011. *Draft Flora of Montana*. Unpublished.	Region 8	MT
Martin, W.C. and C.R. Hutchins. 1980, 1981. *A Flora of New Mexico. Vols. 1,2.* J. Cramer.Vaduz, Germany,	Region 8	NM
McDougall, W.B. 1973. *Seed Plants of Northern Arizona.* Museum of Northern Arizona, Flagstaff, Arizona, 594 pp.	Region 9	AZ
Milburn, S. A., M. Bourdaghs, and J. J. Husveth. *Floristic Quality Assessment for Minnesota Wetlands*. Minnesota Pollution Control Agency, St. Paul, Minn. www.pca.state.mn.us/water/biomonitoring/bio-wetlands.html	Region 5	MN
Missouriplants.com: Photographs and descriptions of the flowering and non-flowering plants of Missouri, USA. http://www.missouriplants.com/	Region 7	MS
Mississippi Trees (2011) Published by The Mississippi Forestry Commission. 337pp.	Regions 4 and 6	MS

Potentially Useful Floras and Field Guides	EPA Region(s) Where ***Potentially*** Applicable	Used by States in NWCA 2011
Mittelhauser, G.H., L.L. Gregory, S.C. Rooney, J.E. Weber. 2010. *The Plants of Acadia National Park*. University of Maine Press. Orono, Maine, 594 pp.	Region 1	ME
Mohlenbrock, R. 2002. *Vascular Flora of Illinois*. Southern Illinois University Press, Carbondale & Edwardsville, Illinois.	Region 5	IL, IN
Mohlenbrock, R .H. 1986. *Guide To The Vascular Flora Of Illinois. Southern Illinois* University Press, Carbondale and Edwardsville, Illinois. 507 pp.	Region 5	IL
Moore, D.M. and J.E. Grant. 2007. *Trees of Arkansas*. Arkansas Forestry Commission.	Region 6	AR, LA
Natural Resources Database. 2011. Checklist of Flora in China Camp SP. http://www.nrdb.org/checklistsearchresults.asp	Region 9	CA
Nelson, G. 1996. *The shrubs and woody vines of Florida*. Pineapple Press, Inc., Sarasota, Florida.	Region 4	FL, MS
Nelson, G. 1994. *The trees of Florida*. Pineapple Press, Inc., Sarasota, Florida.	Region 4	FL
Newcomb, Lawrence. 1977. *Newcomb's Wildflower Guide*. Little, Brown, and Company. New York.	Regions 1, 2, 3, and 5	IL, IN, KY, MI, NY, OH, TN, WI
Newcomb, Lawrence. 1989. *Newcomb's Wildflower Guide*. Little, Brown, and Company. New York, 490 pp.	Regions 1, 2, 3, and 5	MN, NH , PA
Neyland, R. 2009. *Wildflowers of the Coastal Plain: A Field Guide.* Louisiana State University Press, 352 pp.	Regions 4 and 6	LA, MS
Neyland, R. 2011. *A Field Guide to the Ferns and Lycophytes of Louisiana*. Louisiana State University Press, 104 pp.	Region 6	LA
NOAA. 2010. *Selected Plants of Coastal Mississippi and Alabama Grand Bay and Weeks Bay* Nerr. 160 pp.	Region 4	MS
Pojar, J., and A. MacKinnon. 1994. *Plants of the Pacific Northwest Coast: Washington, Oregon, British Columbia & Alaska*. Lone Pine Publishing. Redmond, Washington. 528 pp.	Region 10	OR, WA
Radford, A.E., H.A. Ahles, and C.R. Bell. 1968. *Manual of the Vascular Flora of the Carolinas*. University of North Carolina Press. Chapel Hill, North Carolina.	Regions 4 and 6	AL, AR, GA, LA, NC, SC, TX
Rothrock, P.E. 2009. *Sedges of Indiana and the Adjacent States: The Non-Carex Species*. Indiana Academy of Science. 271 pp.	Region 5	OH
San Luis National Wildlife Refuge Plant Species List	Region 9	CA
San Pablo National Wildlife Refuge Plant Species List	Region 9	CA
Sacramento Regional County Sanitation District Plant List: http://www.srcsd.com/buffer-plant.php. Accessed 2011	Region 9	CA
Shaw, R.J. 1989. *Vascular Plants of Northern Utah: An Identification Manual*. Utah State University Press, Logan, Utah. (note field crew indicated 1982 copyright, but I could not find a citation with that date).	Region 8	UT
Springer, J.D., M.D. Daniels, and M. Nazaire. 2009. *Field Guide to Forest and Mountain Plants of Northern Arizona: From the Mogollon Rim and White Mountains North*. Ecological Restoration Institution Northern Arizona University, Flagstaff, AZ, 649 pp.	Region 9	AZ

Potentially Useful Floras and Field Guides	EPA Region(s) Where ***Potentially*** Applicable	Used by States in NWCA 2011
Standley. 2011. *Field Guide to Carex of New England*. Special Publication of the New England Botanical Club. 182.pp.	Region 1	NH
Strausbaugh, P. D. and E. L Core. 1978. *Flora of West Virginia*, Second Edition. Seneca Books, Morgantown, West Virginia. 1079 pp.	Region 2	WV
Stutzenbaker, C.D. 1999. *Aquatic and Wetland Plants of the Western Gulf Coast*. Texas Parks and Wildlife Press (Distributed by University of Texas Press). 465 pp.	Regions 4 and 6	MS
Stutzenbaker, C.D. 2010. *Aquatic and Wetland Plants of the Western Gulf Coast.* Texas A&M University Press. College Station, Texas. 468 pp.	Regions 4 and 6	LA, MS, TX
Swink, Floyd and Gerould Wilhelm. 1994. *Plants of the Chicago Region*. 4th Ed. Indiana Academy of Science. Indianapolis.	Region 5	IL, IN, WI
Taylor, W.K. 1998*. Florida wildflowers in their natural communities.* University Press of Florida, Gainesville, Florida.	Region 4	FL, Ga
Tiner, R. W. 2009. *Field Guide to Tidal Wetland Plants of the Northeastern United States and Neighboring Canada*. The University of Massachusetts Press. Amherst, Massachusetts.	Regions 1 and 2	NH
Tiner, R. W. 1993. *Field Guide to Coastal Wetland Plants of the Southeastern United States*. The University of Massachusetts Press. Amherst, Massachusetts.	Region 4 and 5	AL, LA, MS, TX
Tobe, J.D., K.C. Burks, R.W. Cantrell, M.A. Garland, M.E. Sweeney, D.W. Hall, P.Wallace, G. Anglin, G. Nelson, J.R. Cooper, B. Bickner, K. Gilbert, N. Aymond, K. Greenwood, N. Raymond. 1998. *Florida wetland plants: an identification manual.* Department of Environmental Protection, Tallahassee, Florida.	Region 4	FL, GA, MS
Tueten, J. and G. Tueten. 1993. *Wildflowers of Houston*. Rice University Press, Houston, Texas, 309 Pp.	Region 6	TX
Turner, M. and P. Gustafson. 2002. *Wildflowers of the Pacific Northwest*. Timber Press, Portland, Oregon, 511 pp.	Region 10	ID
USDA, NRCS (U.S. Department of Agriculture, Natural Resources Conservation Service). 2011. The PLANTS Database (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.	All	CA, MD, NC, VA
Van Bruggen, T. 1985. *The Vascular Plants of South Dakota*. Iowa State University Press. Ames, Iowa, 476 pp.	Region 8	SD
Voss, Edward G. 1972, 1985, 1996. *Michigan Flora: A Guide to the Identification and Occurrence of the Native and Naturalized Seed-plants of the State. Part I: Gymnosperms and Monocots. Part II: Dicots (Saururaceae-Cornaceae). Part III: Dicots (Pyrolaceae-Compositae*). Cranbrook Institute of Science and University of Michigan Herbarium.	Region 5	IN, MI, MN, WI
Washington Department of Ecology. 2001. *An Aquatic Plant Identification Manual for Washington's Freshwater Plants*. Ecology Publication # 01-10-032.	Region 10	WA
Weakley A. S. 2007. *Flora of the Carolinas, Virginia, Georgia, and Surrounding Areas.* Working Draft of 11 Jan 2007, University of North Carolina Herbarium. Chapel Hill, North Carolina.	Regions 3 and 4	NC

Potentially Useful Floras and Field Guides	EPA Region(s) Where ***Potentially*** Applicable	Used by States in NWCA 2011
Weakley, A. S. 2011. *Flora of the Southern and Mid-Atlantic States.* Working Draft of 15 May 2011. University Of North Carolina Herbarium, North Carolina Botanical Garden. Chapel Hill, North Carolina. 1072 pp.	Regions 3 and 4	NC, WV
Weber, W.A. and R.C. Wittmann. 2001. *Colorado Flora, Eastern Slope,* Third Edition. University Press of Colorado, Boulder, Colorado, 521 pp.	Region 8	CO
Weber, W.A. and R.C. Wittmann. 2001. *Colorado Flora Western Slope*, Third Edition. University Press of Colorado, Boulder, Colorado.	Region 8	CO
Weishaupt, C.G. 1971. *Vascular Plants of Ohio: A Manual for Use in Field and Laboratory.* 3rd ed. Kendall Hunt Publishing Co., Dubuque, IA. 292 pp.	Region 5	OH
Welsh, S.L., N.D. Atwood, S. Goodrich, L.C. Higgins, eds. 1993. *A Utah Flora*. Second Edition. Brigham Young University Press. Provo, Utah. 986 pp. (Is this the correct citation?)	Region 8	UT
Whitson, T.D., L.C. Burrill, S.A. Dewey, D.W. Cudney, B.E. Nelson, R.D. Lee, and R. Parker. 1992. *Weeds of the West*. University of Wyoming.	Regions 8, 9, and 10	UT, WA
Wilson, B.L., R. Brainerd, D. Lytjen, B. Newhouse, and N. Otting. 2008. *Field Guide to the Sedges of the Pacific Northwest*. Oregon State University Press. Corvallis, Oregon, 432 pp.	Region 10	ID, OR, WA
Wunderlin, R.P. 1998. *Guide to the vascular plants of Florida*. University Presses of Florida, Gainesville, Florida.	Region 4	FL
Yatskeivych, George. 1999. *Steyermark's Flora of Missouri, Volume 1*. Missouri Department of Consevation Missouri Botanical Garden Press. Jefferson City and St. Louis, Missouri, 991 pp.	Region 7	MO
Yatskeivych, George. 2006. *Steyermark's Flora of Missouri, Volume 2*. Missouri Botanical Garden Press. St. Louis, Missouri, 1200 pp.	Region 7	MO

APPENDIX E: 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.
    a) Lay the specimen flat and avoid overlapping plant parts.
    b) Spread leaves, flowers, and fruits so they can be easily observed from different perspectives.
    c) Show upper and lower surfaces of leaves and flowers.
    d) If possible, arrange material so some flowers have the blossom open, and some flowers and fruits appear in longitudinal and transverse views.
    e) Multiples of smaller plants of the same species should be pressed together on one sheet.
    f) For large specimens, bend stems sharply into a V or N shape so they fit within the press frame. Avoid curving or twisting stems.
    g) 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 Voucher filled in, while unknown samples will have Unknown Species filled in.

Plant Sample ID Number: NWCA Site Number-Plant collection number. Plant collection numbers for samples are assigned consecutive numbers depending on the specimen type (unknown specimens are prefaced with the letter U and QA specimens are prefaced with the letter Q) for each site beginning with one. For example, the sample number for the 14th unknown specimen collected at NWCA16-9999 would be NWCA16-9999-U14.

Visit Number: Indicates whether it was the first visit (1) or a repeat visit (2). Most sites are only visited once.

Collection Date: Date is numerical: month, day, year, e.g. 06/14/2016.

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 (e.g., Carex sp. 1) if unknown.

Collector(s) name: 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.

Habitat: The type of plant community or setting where the plant is growing. (e.g., such as 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).

Growth habit: Describes key features of the plant such as growth form (tree, shrub, vine, herb), approximate height, longevity (annual, biennial, perennial), clonal, rhizomatous, tussock-forming, etc. Lists any characteristics of the plant which may be lost upon drying, such as flower/fruit color, fragrance, and leaf orientation.

Drying Plant Specimens

Pressed plant specimens should be thoroughly dried before removing them from the presses. Once dry, remove specimens from the presses.

- 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 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.
- Periodically tighten the straps on the press as the specimens dry and shrink to maintain pressure on the press.
- 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 label
- Have an acid free fragment envelop (where necessary for seeds and flowers)

APPENDIX F: USGS PROCEDURE FOR ANALYSIS OF ALGAL TOXINS (OGRL-SOP-5400)

Attached as separate PDF file

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