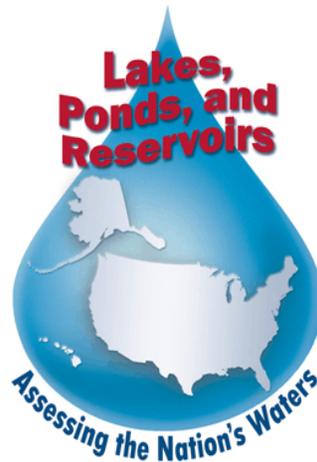




United States Environmental Protection Agency  
Office of Water  
Washington, DC  
EPA 841-B-16-004

# National Lakes Assessment 2017 Laboratory Operations Manual

Version 1.1, May 2017



## NOTICE

The intention of the National Lakes Assessment 2017 (NLA 2017) is to provide a comprehensive assessment for lakes, ponds, and reservoirs across the United States. The complete documentation of overall project management, design, methods, standards, and Quality Assurance/Quality Control measures, is contained in companion documents, including:

National Lakes Assessment 2017: Quality Assurance Project Plan (QAPP) (EPA 841-B-16-003)

National Lakes Assessment 2017: Site Evaluation Guidelines (SEG) (EPA 841-B-16-001)

National Lakes Assessment 2017: Field Operations Manual (FOM) (EPA 841-B-16-002)

This document (Laboratory Operations Manual) contains information on the methods for analyses of the samples for nine indicators: algal toxins (cylindrospermopsin and microcystins), atrazine screen, bacteria (*E. coli*), benthic macroinvertebrates, phytoplankton, sediment chemistry (contaminants, total organic carbon, and grain size), water chemistry and chlorophyll *a*, and zooplankton) to be collected during the project, quality assurance objectives, sample handling, and data reporting. Dissolved gases and fish eDNA analysis are also included as part of the NLA and those methods are available separately. The NLA laboratory methods are based on guidelines developed by federal agencies and methods employed by several key states that were involved in the planning phase of this project. Methods described in this document are to be used specifically in work relating to the NLA 2017. All project cooperator laboratories must follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. Details on specific methods for site evaluation and sampling can be found in the appropriate companion document. Revision history information is found in the associated QAPP document.

### **The suggested citation for this document is:**

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## LIST OF ACRONYMS

A	absorbance
Al	Aluminum
ANOVA	analysis of variance
ANC	acid neutralizing capacity
AV	assistance visit
Ca	calcium
CH <sub>4</sub>	methane
Cl	chloride
CO <sub>2</sub>	carbon dioxide
COB	close of business
CRM	certified reference manual
CV	coefficient of variation
DI	deionized
DO	dissolved oxygen
DOC	dissolved organic carbon
ELISA	enzyme-linked immunosorbent assay
EMAP	Environmental Monitoring and Assessment Program
USEPA	United States Environmental Protection Agency
EtOH	ethyl alcohol
FOM	field operations manual
HDPE	high density polyethylene
H <sub>2</sub> SO <sub>4</sub>	sulfuric acid
HNO <sub>3</sub>	nitric acid
HQ	Headquarters
IBD	ion balance difference
IM	information management
IT	information technology
K	potassium
LCS	laboratory control sample
LOM	laboratory operations manual
LRL	lower reporting limit
Mg	magnesium
MS	matrix spike
MSD	matrix spike duplicate
MDL	method detection limit
MQO	measurement quality objective
MPCA	Minnesota Pollution Control Agency
MPN	most probable number
MRL	Mercury Research Laboratory
N	Nitrogen
NARS	National Aquatic Resource Surveys
NLA	National Lakes Assessment
N <sub>2</sub> O	nitrous oxide
Na	sodium
NH <sub>3</sub>	ammonia
NIST	National Institute of Standards

NO <sub>3</sub>	nitrate
NO <sub>2</sub>	nitrite
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PD	percent difference
PDE	percent difference in enumeration
PSE	percent sorting efficiency
PT	proficiency tests
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 Plans
RL	Reporting Limit
RMSE	root mean square error
RO	reverse osmosis
RPD	relative percent difference
RSD	relative standard deviation
S	standard deviation
SEG	site evaluation guidelines
SiO <sub>2</sub>	silica
SO <sub>4</sub>	sulfate
SOPs	standard operating procedures
SRM	standard reference material
TMB	tetramethylbenzidine
TN	total nitrogen
TOC	total organic carbon
TOCOR	Task Order's Contracting Officer's Representative
TP	total phosphorus
USGS	United States Geological Survey
WRS	Willamette Research Station

# NATIONAL LAKES ASSESSMENT 2017 LABORATORY OPERATIONS MANUAL

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The U.S. Environmental Protection Agency (USEPA), in partnership with state and tribal organizations, has designed the National Lakes Assessment (NLA) 2017 to assess the condition of lakes, ponds and reservoirs (referred to collectively as lakes throughout the document) in the United States. The NLA is one in a series of National Aquatic Resource Surveys (NARS) conducted to provide the public with a comprehensive assessment of the condition of waters in the U.S.

This manual contains procedures for laboratory analysis of samples collected from lakes throughout the lower 48 states of the United States and Alaska. The purposes of this manual are to:

- 1) document the standardized sample processing and analysis procedures used in the various laboratories for the NLA 2017; and
- 2) provide guidance for data quality and a performance-based method approach to obtain comparable results across all participating laboratories.

Detailed laboratory procedures are described for the following indicators: algal toxins, *E. coli* bacteria, benthic macroinvertebrates, phytoplankton, sediment chemistry (contaminants, total organic carbon, and grain size), atrazine screen, water chemistry, chlorophyll *a*, and zooplankton. Two indicators are research indicators, dissolved gases and fish eDNA, and will be completed in collaboration with the USEPA's Office of Research and Development.

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 NLA project management team (USEPA Office of Water).

## 1.0 GENERAL LABORATORY GUIDELINES

### 1.1 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 laboratory shall follow its institutional or organizational requirements for instrument maintenance. Specific laboratory qualification documentation required for analysis is contained in the Quality Assurance Project Plan (QAPP).

### 1.2 Roles and Contact Information

**Table 1-1** presents contact information for the key personnel associated with NLA 2017. The **USEPA Headquarters Project Management Team** consists of the Project Leader, Alternate Project Leaders, and Project QA Lead. 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 **Contractor Logistics Coordinator** and the **NARS Information Management (IM) Coordinator** track the location of each NLA 2017 sample that involves laboratory processing. These coordinators will be the laboratories main point of contact in regards to sample tracking.

**Table 1-1 Contact information.**

Title	Name	Contact Information
<b>USEPA HQ Project Lead</b>	Amina Pollard, OW	<a href="mailto:pollard.amina@epa.gov">pollard.amina@epa.gov</a> 202-566-2360
<b>USEPA HQ Project QA Coordinator</b>	Sarah Lehmann, OW	<a href="mailto:lehmann.sarah@epa.gov">lehmann.sarah@epa.gov</a> 202-566-1379
<b>USEPA HQ Logistics Lead</b>	Brian Hasty, OW	<a href="mailto:hasty.brain@epa.gov">hasty.brain@epa.gov</a> 202- 564-2236
<b>USEPA HQ Laboratory Review Coordinator</b>	Kendra Forde, OW	<a href="mailto:forde.kendra@epa.gov">forde.kendra@epa.gov</a> 202-566-0417
<b>Information Management (IM) Center Coordinator</b>	Marlys Cappaert, SRA International Inc.	<a href="mailto:cappaert.marlys@epa.gov">cappaert.marlys@epa.gov</a> 541-754-4467 541-754-4799 (fax)
<b>Contractor Logistics Coordinator</b>	Chris Turner, GLEC	<a href="mailto:cturner@glec.com">cturner@glec.com</a> 715-829-3737

### 1.3 Sample Tracking

Samples are collected by a large number of field crews during the index period (June through September). The actual number of lakes sampled on a given day will vary widely during this time. Field crews submit electronic and/or paper forms when they have shipped samples and the NARS IM Center inputs each sample into the NARS IM database. Processing laboratories can track sample shipment from field crews by accessing the information uploaded in the NARS IM database by way of the NARS SharePoint site. Participating laboratories and all pertinent personnel will be given access to the NARS SharePoint site, where they can acquire site and sample status information. This will include check-in

and batching information on samples that have been shipped to the batch laboratory by field crews (either by overnight shipment for perishable samples or batch shipments for preserved samples). The NARS IM Center provides laboratories with spreadsheets of samples that the batch lab or field crews (as appropriate) have sent to them. Upon sample receipt, the analysis laboratory must immediately complete and email the sample tracking spreadsheet (containing the sample login and sample condition information) to the IM Center Coordinator for confirmation of sample receipt. Each laboratory will make arrangements with the USEPA HQ Laboratory Review Coordinator for access to the NARS SharePoint site and the NARS IM Center Coordinator, both listed above, to ensure the process of sample check-in has been organized before samples begin to arrive.

When the samples arrive from the field crews, laboratories also receive tracking forms in the shipment (refer to the NLA 2017 FOM). These forms list the samples included in the shipment. Laboratory personnel must cross check the forms with the samples received to verify that there are not any inconsistencies. If any sample is missing or damaged, contact the IM Center Coordinator and/or Contractor Logistics Coordinator immediately. For state laboratories conducting analyses in their own laboratories, a state sample tracking spreadsheet is available from EPA.

## 1.4 Reporting

All laboratories must provide data analysis information to the HQ Project Management Team and the NARS IM Center by May 1, 2018 or earlier as stipulated in contractual agreements. These reports must include the following information and be reported in the data templates available separately from EPA.

- Sample Type (indicator)
- Site ID (ex: NLA17\_AL-10007)
- Sample ID (ex: 999000)
- Pertinent information to the indicator
- Metadata for all fields

The submitted file name must state the following:

- Indicator name (ex: microcystins)
- Date of files submission by year, month, and day (ex: 2017\_11\_01)
- Laboratory name (ex: MyLaboratory)

Combined, the file name would look as follows: Microcystin\_2017\_11\_01\_MyLaboratory.xlsx

As specified in the QAPP, remaining sample material and specimens must be maintained by the USEPA's designated laboratory or facilities as directed by the NLA 2017 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 USEPA Project Leader. Deliverables from contractors and cooperators, including raw data, are permanent as per USEPA Record Schedule 258. USEPA's project records are scheduled 501 and are also permanent.

## 2.0 LABORATORY QUALITY CONTROL

As part of the NLA 2017, field samples will be collected at each assessment site unless otherwise specified. These samples will be sent to laboratories cooperating in the assessment. To ensure quality, each Project Cooperator laboratory analyzing samples from the NLA 2017 will participate in a laboratory verification process. All Project Cooperator laboratories will follow these guidelines.

No national program of accreditation for laboratory processing for most of our indicators currently exists. For this reason, a rigorous program of laboratory evaluation and verification has been developed to support the NLA 2017.

Given the large number of laboratories participating in the NLA 2017, it is not feasible to perform an assistance visit<sup>a</sup> (AV) on each of these laboratories. An AV would include an on-site visit to the laboratory lasting at least a day. As a result, the USEPA Headquarters Project Management Team will conduct remote review of laboratory certifications and accreditations of all laboratories and an inter-laboratory comparison will be performed between some laboratories (mainly for biological indicators). This process is called laboratory verification and is conducted before sample processing and analysis begins. If issues arise from the remote review or inter-laboratory comparison that cannot be resolved remotely then an on-site visit to the laboratory will be performed. The NLA 2017 Project Management Team believes this approach meets the needs of this assessment and can ensure quality control on data generated by the participating laboratories. General information is provided here and more specifics are provided in **Sections 2.1** and **2.2**.

Competency. To demonstrate its competency, the laboratory shall provide analyte and matrix specific information to the USEPA; or information specific to the relevant biological indicator. The USEPA 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, a curriculum vita for taxonomists, and experience with analyses that are the same or similar to the requirements of this method.
- Demonstration of competency with sediment and water chemistry 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, each laboratory shall provide the USEPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), QAPPs, and applicable SOPs.

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<sup>a</sup> The evaluation and verification of the laboratories is being considered as a form of an AV rather than an audit because the evaluation and verification phase is designed for the laboratories to demonstrate competency of performance and for the EPA HQ Project Management Team to provide guidance to the laboratories rather than as “inspection” as in a traditional audit.

To demonstrate its ongoing commitment, the person in charge of quality issues for the participating laboratory shall sign the NLA QAPP Certification Page, which will be maintained at the USEPA in a quality assurance file.

## 2.1 Laboratory Verification Process/Technical Assessment

Procedural review and assistance personnel are trained to the specific implementation and data collection methods detailed in this NLA 2017 LOM. Laboratory evaluation and verification reinforces the specific techniques and procedures for both field and laboratory applications. A remote evaluation and verification procedure has been developed for performing assessment of all laboratories.

Laboratory evaluation and verification process 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 and verification 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 NLA 2017 project, we have requested that each participating laboratory provide the following documentation:

- The laboratory's Quality Manual, (QMP) or similar document.
- SOPs for each analysis to be performed.
- Long term Method Detection Limits (MDLs) for each instrument used and Demonstration of Capability for each analysis to be performed.
- A list of the laboratory's accreditations and certifications (e.g. NELAP, ISO, etc.), if any.
- Results from Proficiency Tests (PT) for each analyte to be analyzed under the NLA project.
- Relevant curriculum vitae and documents demonstrating previous survey participation.

If a laboratory has clearly documented procedures for sample receiving, storage, preservation, preparation, analysis, and data reporting; has successfully analyzed PT samples (if required by the USEPA, the USEPA 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 the 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 assistance or fails to produce the requested documentation.

All laboratory personnel responsible for quality must sign the NLA 2017 QAPP signature page.

In addition, all laboratories must sign a Laboratory Signature Form (in **APPENDIX A: LABORATORY REMOTE EVALUATION AND VERIFICATION FORMS**) indicating that they will abide by the following:

1. Utilize procedures identified in the NLA 2017 Laboratory Operations Manual (or equivalent). If using equivalent procedures, please provide procedural manual to demonstrate ability to meet the required MQOs.
2. Read and abide by the NLA 2017 Quality Assurance Project Plan (QAPP) and related SOPs.

3. Have an organized IT system in place for recording sample tracking and data analysis.
4. Provide data to the USEPA using the template provided in the Laboratory Operations Manual.
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, 2018 or as otherwise negotiated with the USEPA.
6. Participate in a laboratory technical assessment or audit if requested by the USEPA NLA staff (this may be a conference call or on-site audit).

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

1. Use taxonomic standards outlined in the NLA 2017 Laboratory Manual.
2. Participate in taxonomic reconciliation exercises during the field and data analysis season, which include conference calls and other laboratory reviews (see more below on Inter-laboratory comparison).

## **2.2 Inter-laboratory Comparison**

An inter-laboratory investigation is being implemented for the laboratories performing analysis on benthic macroinvertebrates, phytoplankton, and zooplankton data for the NLA 2017. This process is defined as an inter-laboratory comparison since the same protocols and method will be used by all participating laboratories as described in this manual. No on-site assistance visit is envisioned for these laboratories unless the data submitted and reviewed by the USEPA does not meet the requirements of the inter-laboratory comparison described.

## 3.0 ALGAL TOXIN: CYLINDROSPERMOPSIN IMMUNOASSAY PROCEDURE

This chapter describes an immunoassay procedure that measures concentrations of total cylindrospermopsin in water samples. In applying the procedure, the laboratory uses Abraxis' Cylindrospermopsin Test Kits ("kits"). Each kit is an enzyme-linked immunosorbent assay (ELISA) for the determination of cylindrospermopsin in water samples.

Cold cylindrospermopsin samples will be shipped on ice from the field crews to the contract batching laboratory. The contract batching laboratory will freeze samples and send the batched samples to the analysis laboratory in coolers on dry ice. Samples will arrive in the analysis laboratory frozen and they can be held in a freezer for several weeks. Cylindrospermopsin analysis laboratories will need to process samples in accordance with the time frame outlined in contractual agreements.

The procedure is an adaption of the instructions provided by Abraxis for determining total cylindrospermopsin concentrations using its ELISA kits.<sup>b</sup> For freshwater samples, the procedure's reporting range is 0.1 µg/L to 2.0 µg/L, although, theoretically, the procedure can detect, not quantify, cylindrospermopsin concentrations as low as 0.05 µg/L. For samples with concentrations higher than 2.0 µg/L of cylindrospermopsin, the procedure includes the necessary dilution steps.

### 3.1 Definitions and Required Personnel Qualifications

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

#### 3.1.1 Definitions

The following terms are used throughout the procedure:

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

**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 0.1 µg/L to a maximum value of 2.0 µg/L. Values outside the range are handled as follows. If the value is:

- < 0.05 µg/L, then the laboratory reports the result as being non-detected (" $<0.10$  µg/L").
- Between 0.05 µg/L and the reporting limit of 0.1 µg/L (i.e.,  $>0.05$  µg/L and  $<0.1$  µg/L), the laboratory should record the value, but assign a Quality Control (QC) code to the value (i.e., DATA\_FLAG=J).
- 2.0 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:

### Equation 3.1 Standard deviation

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

where  $n$  is the number of replicate samples,  $A_i$  is the absorbance measured for the  $i^{\text{th}}$  replicate. Per **Section 3.5.3**, 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:

### Equation 3.2 Percent (%) coefficient of variation

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

**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 (0.05 µg/L). 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 0.1 µg/L, at which the *measured* value of the analyte can be reported with confidence. Also see “Sample-Specific Detection Limit” below.

**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 3.5.3**, 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 provide the CV in their outputs, the procedure presents the quality control requirement in terms of %CV instead of RSD.

**Reporting Limit:** For undiluted freshwater samples, the reporting limit is 0.1 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 limit equal to the method’s detection limit of 0.05 µg/L. For diluted samples, the sample-specific detection limit will be the product of the method’s detection limit of 0.05 µg/L and the dilution factor. Typical values for the dilution factor will be 10 or 100.

### 3.1.2 Personnel Qualifications

**Laboratory Technician:** This procedure may be used by any laboratory technician who is familiar with the NLA QAPP, and this procedure in the NLA 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 USEPA 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 the NLA. 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.

## 3.2 Precautions

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., laboratory 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 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.

## 3.3 Equipment/Materials

The procedures require the following equipment and information:

- Abraxis Cylindrospermospin ELISA (Microtiter) Test Kit, Product # 522011 (see items in **Section 3.5.2**).
- Adhesive Sealing Film (Parafilm) for Micro Plates: Used to cover plates during incubation.
- Data Template – See **Figure 3.1**.
- Distilled or Deionized Water: For diluting samples when necessary.
- ELISA evaluation software.
- 2 glass scintillation, LC, vials (20 mL).
- Multichannel Pipette & Tips: An 8-channel pipette is used for this method. Familiarity of the use of the multichannel pipette is necessary to achieve reliable results. Practice with water if you have never used this before.
- 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 (such as Metertech, Model M965 AccuReader): Complete with Metertech PC Mate software for operation of machine. This machine reads the microtiter plates.
- Project Quality Control Samples.
- Reagent Reservoirs (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).

### 3.4 Sample Receipt

Because USEPA 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 to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** of the manual for contact information).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
  - a. Verify that the sample IDs in the shipment match those recorded on the sample tracking form.
  - b. Record the information in **Table 3.3-1** for the NARS IM Team, 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. *W*: Sample is warm (>8°), record the temperature in the comment field, and perform the assay
  - c. If any sample is damaged or missing, contact the USEPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).
3. Store samples in the freezer until sample preparation begins.
4. Maintain the sample tracking forms with the samples.

**Table 3.3-1 Cylindrospermopsin login: required data elements.**

FIELD	FORMAT	DESCRIPTION	
LABORATORY ID	text	Name or abbreviation for QC laboratory	
DATE RECEIVED	MMDDYY	Date sample was received by laboratory	
SITE ID	text	NLA 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°)
		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	

### 3.5 Procedure

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

#### 3.5.1 Sample Preparation

For each frozen sample (500 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 500 ml sample.
  - b. Thaw the sample to room temperature (approximately 25° C). Swirl the sample to check for ice crystals. At this temperature, no ice crystals should be present in the sample.
  - c. Shake well to homogenize the sample, then transfer 10 mL to an appropriately labeled clean 20 mL glass vial.
3. Second freeze-thaw cycle:
  - a. Freeze the vial.
  - b. Keep the large sample bottle (from the 500 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.

#### 3.5.2 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 (7) referenced in this procedure as follows with the associated concentration:
    - S0: 0 µg/L
    - S1: 0.05 µg/L
    - S2: 0.1 µg/L,
    - S3: 0.25 µg/L
    - S4: 0.5 µg/L
    - S5: 1.0 µg/L
    - S6: 2.0 µg/L
  - Kit Control (KC): 0.75 µg/L
  - Sample Diluent (distilled or deionized water)
  - Cyldrospermospin-HRP conjugate Soution (vortex before use)
  - Antibody solution (rabbit anti-Cylindrospermopsin)
  - Wash Solution 5X Concentrate
  - Substrate (Color) Solution
  - Stop Solution
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 store in the refrigerator (4-8 °C).
  6. Prepare a negative control (NC) using distilled or deionized water
  7. The standards, controls, antibody solution, enzyme conjugate, color solution, and stop solutions are ready to use and do not require any further dilutions.
  8. Dilute the wash solution with distilled or 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 distilled or deionized water). Mix thoroughly. Set aside the diluted solution to wash the microtiter wells later.
  9. Handle the stop solution containing diluted H<sub>2</sub>SO<sub>4</sub> (sulfuric acid) with care.

### 3.5.3 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 3.1**) to use as reference when loading the standards, controls, and samples as described in the next step. Templates contain rows, labeled with a marking pen, of strips of 8 wells that snap into the blank frame. (If the laboratory wishes to use a different

template, provide a copy to the USEPA HQ Laboratory Review Coordinator for approval prior to first use. (See Section 1.2 of the manual for contact information.)

4. Using the 100- $\mu$ L pipette, add 50  $\mu$ L, each, of the standards, controls, and samples to the appropriate wells in the plate. Place all seven standards (0.00, 0.05, 0.10, 0.25, 0.50, 1.0 and 2.0  $\mu$ g/L), the kit control (0.75  $\mu$ L), and negative control, in pairs (duplicate), starting in the well in the upper left-hand corner of the kit as shown in **Figure 3.1**. Verify that the software displays the same template or make any necessary corrections.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S4	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	S6	U2	U6	U10	U14	U18	U22	U26	U30	U34	U38
F	S2	S6	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	KC	U3	U7	U11	U15	U19	U23	U27	U31	U35	U39

**Figure 3.1** Cylindrospermopsis: sample template.

Key:

S0-S6 = Standards;

KC = Control supplied with Kit (i.e., Kit Control);

NC = Negative Control (Laboratory Reagent Blank);

U = Unknown (sample collected by the field crew);

5. Add 50  $\mu$ L of the conjugate solution to each well using the multi-channel pipettor and a reagent reservoir. Add 50  $\mu$ L of the cylindrospermopsis 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 45 minutes.
9. After 45 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 four times with 250  $\mu$ L 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  $\mu$ L of substrate/ color 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 30-45 minutes.

15. After 30-45 minutes, remove the Parafilm, add 100 µL of stop solution to the wells using the multi-channel pipette and reagent reservoir in the same sequence as the substrate solution.
16. Use a 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.
17. Dispose of solution in plates in a laboratory sink. Rinse plates and sink with water to dilute the weak acid present.
18. 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-S6 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 Cylindrospermopsin (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 +/- 0.15 ppb. That is, results must be between 0.60 and 0.90.
  - 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 3.5.4**.
    - ii. The %CV  $> 15\%$  between the duplicate absorbance values for a sample.
19. Record the results, even if the data failed the quality control requirements in #18b, for each well in the USEPA's data template. The required entries are for the following columns:
  - a. **TYPE** should be one of the following codes: S0-S6 for standards; KC or NC, 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 0.05 µg/L if the sample is undiluted. See **Section 3.5.4** 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 0.1 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 18 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).

### 3.5.4 Dilutions (if needed)

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

#1

1:10 dilution

- a. Add 900 µL of distilled or deionized water to a clean vial. (Note: Dilutions may also be made using the kit's diluent rather than distilled or deionized 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 0.05 µg/L by 10 to obtain the sample-specific detection limit of .5 µg/L.

#2

1:100 dilution

- a. Add 3.96 mL of distilled or deionized water to a clean, appropriately labeled glass vial. (Note: Dilutions may also be made using the kit's diluent rather than distilled or deionized 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 the sample again.
- c. Multiply the final concentration and Abraxis' detection limit of 0.05 µg/L by 100 to obtain the sample-specific detection limit of 50 µg/L.

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

## 3.6 Pertinent QA/QC Procedures

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

### 3.6.1 QC Samples

The External QC Coordinator will instruct the QC contractor to provide one or two identical sets of

freshwater PT samples to all participating laboratories. Each set will contain five samples to test the expected range of concentrations in the NLA samples.

For the contract laboratory, the QC contractor will provide the first set to be run with the first set of samples and a second set to be run at the midpoint of the assigned samples. If available, a third set will be run with the final batch of samples. Because most state laboratories will have relatively few samples that can be analyzed using a single kit, the QC contractor will send only one set to each state laboratory.

Each laboratory will run the QC samples following the same procedures used for the other samples. The External QC Coordinator 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.

### 3.6.2 Summary of QA/QC Requirements

Table 4-2 provides a summary of the quality control requirements described in Sections 3.5.2 and 3.5.3. For cylindrospermopsin, the precision for a sample is reported in terms of the percent coefficient of variation (%CV) of its absorbance values. Relative Standard Deviation (RSD) is the same as the %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. Accuracy is calculated by comparing the average concentration of the kit control with the required range (0.75 +/- 0.15).

Table 3-2 Cylindrospermopsin: sample and analysis quality control activities and objectives.

Quality Control Activity	Description and Requirements	Corrective Action
<b>Kit – Shelf Life</b>	Is within its expiration date listed on kit box.	If kit has expired, then discard or set aside for training activities.
<b>Kit – Contents</b>	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.
<b>Calibration</b>	All of the following must be met: <ul style="list-style-type: none"> <li>○ Standard curve must have a correlation coefficient of <math>\geq 0.99</math>;</li> <li>○ Average absorbance value, <math>\bar{A}_0</math>, for S0 must be <math>&gt;0.80</math>; and</li> <li>○ Standards S0-S6 must have decreasing average absorbance values. That is, if <math>\bar{A}_i</math> is the average of the absorbance values for <math>S_i</math>, then the absorbance</li> </ul>	If any requirement fails: <ul style="list-style-type: none"> <li>• Results from the analytical run are not reported.</li> <li>• All samples in the analytical run are reanalyzed until calibration provides acceptable results. At its discretion, the laboratory may consult with USEPA for</li> </ul>

	average values must be: $\bar{A}_0 > \bar{A}_1 > \bar{A}_2 > \bar{A}_3 > \bar{A}_4 > \bar{A}_5 > \bar{A}_6$	guidance on persistent difficulties with calibration.
<b>Kit Control</b>	The average concentration value of the duplicates (or triplicate) must be within the range of 0.75 +/- 0.15 µg/L. That is, results must be between 0.60 and 0.90.	<p>If either requirement fails:</p> <ul style="list-style-type: none"> <li>• Results from the analytical run are not reported</li> <li>• The laboratory evaluates its processes, and if appropriate, modifies its processes to correct possible contamination or other problems.</li> <li>• The laboratory reanalyzes all samples in the analytical run until the controls meet the requirements.</li> </ul>
<b>Negative Control</b>	The values for the negative control replicates must meet the following requirements: <ul style="list-style-type: none"> <li>○ All concentration values must be &lt; 0.1 µg/L (i.e., the reporting limit); and</li> <li>○ One or more concentration results must be nondetectable (i.e., &lt;0.05 µg/L)</li> </ul>	
<b>Sample Evaluations</b>	All samples are run in duplicate. Each duplicate pair must have %CV ≤ 15% between its absorbance values.	<p>If %CV of the absorbance for the sample &gt; 15%, then:</p> <ul style="list-style-type: none"> <li>• Record the results for both duplicates using different start dates and/or start times to distinguish between the runs.</li> <li>• Report the data for both duplicate results using Quality Control Failure flag “QCF”; and</li> <li>• Re-analyze the sample in a new analytical run. <b>No samples are to be run more than twice.</b></li> </ul> <p>If the second run passes, then the data analyst will exclude the data from the first run (which will have been flagged with “QCF”). If both runs fail, the data analyst will determine if either value should be used in the analysis (e.g., it might be acceptable to use data if the CV is just slightly over 15%).</p>
<b>Results Within Calibration Range</b>	All samples are run in duplicate. If both of the values are less than the upper calibration range (i.e., 2.0 µg/L for undiluted samples), then the requirement is met.	If a result registers as “HIGH”, then record the result with a data flag of “HI.” If one or both duplicates register as ‘HIGH,’ then the sample must be diluted and re-run. No samples are to be run more than

		twice. The laboratory reports both the original and diluted sample results.
<b>External Quality Control Sample</b>	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.

## 4.0 ALGAL TOXIN: MICROCYSTIN IMMUNOASSAY PROCEDURE

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"). See also EPA Standard Method 546. Each kit is an enzyme-linked immunosorbent assay (ELISA) for the determination of microcystins and other 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.

Cold microcystin samples will be shipped on ice from the field crews to the contract batching laboratory. The contract batching laboratory will freeze samples and send the batched samples to the analysis laboratory in coolers on dry ice. Samples will arrive in the analysis laboratory frozen and they can be held in a freezer for several weeks. Microcystin analysis laboratories will need to process samples in accordance with the time frame outlined in contractual agreements.

The procedure is an adaption of the instructions provided by Abraxis for determining total microcystins concentrations using its ELISA-ADDA kits.<sup>c</sup> 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, microcystin concentrations as low as 0.10 µg/L. For samples with higher concentrations of microcystins, the procedure includes the necessary dilution steps.

### 4.1 Definitions and Personnel Qualifications

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

#### 4.1.1 Definitions

The following terms are used throughout the procedure:

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

**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 (" $<0.10 \mu\text{g/L}$ ").
- Between 0.10 µg/L and the reporting limit of 0.15 µg/L (i.e.,  $>0.10 \mu\text{g/L}$  and  $<0.15 \mu\text{g/L}$ ), 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.

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<sup>c</sup> 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](http://www.abraxiskits.com/uploads/products/docfiles/278_Microcystin%20PL%20ADDA%20Users%20R120214.pdf).

**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:

**Equation 4.1 Standard deviation**

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

where  $n$  is the number of replicate samples,  $A_i$  is the absorbance measured for the  $i^{\text{th}}$  replicate. Per **Section 4.4.3**, 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:

**Equation 4.2 Percent (%) coefficient of variation**

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

**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 ( $0.1 \mu\text{g/L}$ ). 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  $0.15 \mu\text{g/L}$  at which the *measured* value of the analyte can be reported with confidence. Also see “Sample-Specific Detection Limit” below.

**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 4.4.3**, 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 provide the CV in their outputs, the procedure presents the quality control requirement in terms of %CV instead of RSD.

**Reporting Limit:** For undiluted freshwater samples, the reporting limit is  $0.15 \mu\text{g/L}$ . 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 limits equal to the method’s detection limit of  $0.1 \mu\text{g/L}$ . For diluted samples, the sample-specific detection limit will be the product of the method’s detection limit of  $0.1 \mu\text{g/L}$  and the dilution factor. Typical values for the dilution factor will be 10 or 100.

#### 4.1.2 Personnel Qualifications

**Laboratory Technician:** This procedure may be used by any laboratory technician who is familiar with the NLA QAPP, and this procedure in the NLA 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 USEPA 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 the NLA. 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.

## 4.2 Precautions

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. laboratory 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 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.

### 4.2.1 Equipment/Materials

The procedures require the following equipment and information:

- Abraxis ADDA Test Kit, Product #520011 (see items in **Section 4.4.2**)
- Adhesive Sealing Film (Parafilm) for Micro Plates): Used to cover plates during incubation.
- Data Template – See **Figure 4.1**.
- Distilled or Deionized Water: For diluting samples when necessary.
- ELISA evaluation software
- 2 glass scintillation, LC, vials (20 mL)
- Multichannel Pipette & Tips: An 8-channel pipette is used for this method. Familiarity of the use of the multichannel pipette is necessary to achieve reliable results. Practice with water if you have never used this before.
- 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 (such as Metertech, Model M965 AccuReader): Complete with Metertech PC Mate software for operation of machine. This machine reads the microtiter plates.
- Project Quality Control Samples
- Reagent Reservoirs (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  $\mu$ m filter)

### 4.3 Sample Receipt

Because USEPA 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 to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** of the manual for contact information).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
  - a. Verify that the sample IDs in the shipment match those recorded on the sample tracking form.
  - b. Record the information in **Table 4-1** for the NARS IM Team, 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. *W*: Sample is warm (>8°), record the temperature in the comment field, and perform the assay
  - c. If any sample is damaged or missing, contact the USEPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).
3. Store samples in the freezer until sample preparation begins.
4. Maintain the sample tracking forms with the samples.

**Table 4-1 Microcystins login: required data elements.**

FIELD	FORMAT	DESCRIPTION	
LABORATORY ID	text	Name or abbreviation for QC laboratory	
DATE RECEIVED	MMDDYY	Date sample was received by laboratory	
SITE ID	text	NLA 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°)
		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	

#### 4.4 Procedure

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

##### 4.4.1 Sample Preparation

For each frozen sample (500 mL per sample), the laboratory technician runs it through a freeze-thaw cycle three times to lyse the cells as follows:

20. All cycles: Keep the samples in dark or dimly lit areas (i.e., away from sunlight, but under incandescent lighting is acceptable).
21. First freeze-thaw cycle:
  - a. Start with a frozen 500 ml sample.
  - b. Thaw the sample to room temperature (approximately 25° C). Swirl the sample to check for ice crystals. At this temperature, no ice crystals should be present in the sample.
  - c. Shake well to homogenize the sample, then transfer 10 mL to an appropriately labeled clean 20 mL glass vial.
22. Second freeze-thaw cycle:
  - a. Freeze the vial.
  - b. Keep the large sample bottle (from the 500 mL initial sample) frozen for future use.
  - c. Thaw the sample vial contents to room temperature.
23. 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.

##### 4.4.2 Kit Preparation

The technician prepares the kits using the following instructions:

24. 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.)

25. 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 (either distilled or deionized water)

26. 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.

27. Adjust the microtiter plate, samples, standards, and the reagents to room temperature.

28. 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 store in the refrigerator (4-8 °C).

29. Prepare a negative control (NC) using distilled water

30. The standards, controls, antibody solution, enzyme conjugate, color solution, and stop solutions are ready to use and do not require any further dilutions.

31. Dilute the wash solution with distilled or 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 distilled or deionized water). Mix thoroughly. Set aside the diluted solution to wash the microtiter wells later.

32. Handle the stop solution containing diluted H<sub>2</sub>SO<sub>4</sub> with care.

#### 4.4.3 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.

33. 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.

34. Turn on the computer so that it can control and access the plate reader.

35. Print the template (**Figure 4.1**) to use as reference when loading the standards, controls, and samples as described in the next step. Templates contain rows, labeled with a marking pen, of

strips of 8 wells that snap into the blank frame. (If the laboratory wishes to use a different template, provide a copy to the USEPA HQ Laboratory Review Coordinator for approval prior to first use. (See **Section 1.2** of the manual for contact information.)

36. Using the 100- $\mu$ L pipette, add 50  $\mu$ L, 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  $\mu$ g/L), the kit control (0.75  $\mu$ L), and negative control, in pairs, starting in the well in the upper left-hand corner of the kit as shown in **Figure 4.1**. Verify that the software displays the same template or make any necessary corrections.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S4	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 4.1 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);

37. Add 50  $\mu$ L 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.
38. Place the sealing Parafilm over the wells.
39. 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.
40. Place the plate in an area away from light for 90 minutes.
41. After 90 minutes, carefully remove the Parafilm.
42. 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  $\mu$ L 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.
43. Add 100  $\mu$ L of enzyme conjugate solution to all wells using the multi-channel pipettor.
44. Cover the wells with Parafilm.
45. 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.
46. Place the strip holder in an area away from light for 30 minutes.

47. After 30 minutes, remove the Parafilm, decant, and rinse the wells three times again with 250  $\mu$ L of washing solution as described in step 10.
48. Add 100  $\mu$ L 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.
49. Cover the wells with Parafilm.
50. 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.
51. Place the plate in an area away from light for 20 minutes.
52. After 20 minutes, remove the Parafilm and add 50  $\mu$ L 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.
53. 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.
54. Dispose of solution in plates in a laboratory sink. Rinse plates and sink with water to dilute the weak acid present.
55. 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  $> 0.1 \mu\text{g/L}$ ). 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  $0.75 \pm 0.185$  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 4.4.4**.
    - ii. The %CV  $> 15\%$  between the duplicate absorbance values for a sample.
56. Record the results, even if the data failed the quality control requirements in #55b, for each well in the USEPA's data template. The required entries are for the following columns:

- a. **TYPE** should be one of the following codes: S0-S5 for standards; KC or NC 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 4.4.4** 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 55 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).

#### 4.4.4 Dilutions (if needed)

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

#1

1:10 dilution

- e. Add 900 µL of distilled or deionized water to a clean vial. (Note: Dilutions may also be made using the kit’s diluent rather than distilled or deionized water.)
- f. 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.)
- g. Mix by vortexing.
- h. 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.

#2

1:100 dilution

- d. 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 or deionized water.)
- e. 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 the sample again.

- f. 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.

#### 4.5 Pertinent QA/QC Procedures

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

##### 4.5.1 QC Samples

The External QC Coordinator will instruct the QC contractor to provide one or two identical sets of freshwater PT samples to all participating laboratories. Each set will contain five samples to test the expected range of concentrations in the NLA samples.

For the contract laboratory, the QC contractor will provide the first set to be run with the first set of samples and a second set to be run at the midpoint of the assigned samples. If available, a third set will be run with the final batch of samples. Because most state laboratories will have relatively few samples that can be analyzed using a single kit, the QC contractor will send only one set to each state laboratory.

Each laboratory will run the QC samples following the same procedures used for the other samples. The External QC Coordinator 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.

##### 4.5.2 Summary of QA/QC Requirements

**Table 4-2** provides a summary of the quality control requirements described in **Sections 4.4.2 and 4.4.3**. For microcystin, the precision for a sample is reported in terms of the percent coefficient of variation (%CV) of its absorbance values. Relative Standard Deviation (RSD) is the same as the %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. Accuracy is calculated by comparing the average concentration of the kit control with the required range (0.75 +/- 0.185).

**Table 4-2 Microcystin: sample and analysis quality control activities and objectives.**

Quality Control Activity	Description and Requirements	Corrective Action
<b>Kit – Shelf Life</b>	Is within its expiration date listed on kit box.	If kit has expired, then discard or set aside for training activities.
<b>Kit – Contents</b>	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.

<p><b>Calibration</b></p>	<p>All of the following must be met:</p> <ul style="list-style-type: none"> <li>○ Standard curve must have a correlation coefficient of <math>\geq 0.99</math>;</li> <li>○ Average absorbance value, <math>\bar{A}_0</math>, for S0 must be <math>&gt; 0.80</math>; and</li> <li>○ Standards S0-S5 must have decreasing average absorbance values. That is, if <math>\bar{A}_i</math> is the average of the absorbance values for <math>S_i</math>, then the absorbance average values must be: <math>\bar{A}_0 &gt; \bar{A}_1 &gt; \bar{A}_2 &gt; \bar{A}_3 &gt; \bar{A}_4 &gt; \bar{A}_5</math></li> </ul>	<p>If any requirement fails:</p> <ul style="list-style-type: none"> <li>● Results from the analytical run are not reported.</li> <li>● All samples in the analytical run are reanalyzed until calibration provides acceptable results. At its discretion, the laboratory may consult with USEPA for guidance on persistent difficulties with calibration.</li> </ul>
<p><b>Kit Control</b></p>	<p>The average concentration value of the duplicates (or triplicate) must be within the range of <math>0.75 \pm 0.185 \mu\text{g/L}</math>. That is, results must be between 0.565 and 0.935.</p>	<p>If either requirement fails:</p> <ul style="list-style-type: none"> <li>● Results from the analytical run are not reported</li> </ul>
<p><b>Negative Control</b></p>	<p>The values for the negative control replicates must meet the following requirements:</p> <ul style="list-style-type: none"> <li>○ All concentration values must be <math>&lt; 0.15 \mu\text{g/L}</math> (i.e., the reporting limit); and</li> <li>○ One or more concentration results must be nondetectable (i.e., <math>&lt; 0.10 \mu\text{g/L}</math>)</li> </ul>	<ul style="list-style-type: none"> <li>● The laboratory evaluates its processes, and if appropriate, modifies its processes to correct possible contamination or other problems.</li> <li>● The laboratory reanalyzes all samples in the analytical run until the controls meet the requirements.</li> </ul>
<p><b>Sample Evaluations</b></p>	<p>All samples are run in duplicate. Each duplicate pair must have <math>\%CV \leq 15\%</math> between its absorbance values.</p>	<p>If <math>\%CV</math> of the absorbances for the sample <math>&gt; 15\%</math>, then:</p> <ul style="list-style-type: none"> <li>● Record the results for both duplicates using different start dates and/or start times to distinguish between the runs.</li> <li>● Report the data for both duplicate results using Quality Control Failure flag “QCF”; and</li> <li>● Re-analyze the sample in a new analytical run. <b>No samples are to be run more than twice.</b></li> </ul> <p>If the second run passes, then the data analyst will exclude the data from the first run (which will have been flagged with “QCF”). If both runs fail, the data analyst will determine if either value should</p>

		be used in the analysis (e.g., it might be acceptable to use data if the CV is just slightly over 15%).
<b>Results Within Calibration Range</b>	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 a result registers as “HIGH”, then record the result with a data flag of “HI.” If one or both duplicates register as ‘HIGH,’ then the sample must be diluted and re-run. No samples are to be run more than twice. The laboratory reports both the original and diluted sample results.
<b>External Quality Control Sample</b>	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.

## 5.0 BACTERIA (*E. COLI*) METHOD

This method describes the analysis of water samples for the enumeration of *Escherichia coli* (*E. coli*) using *E. coli* using the IDEXX Quanti-Tray/2000 System with Colilert reagent (Standard Method, 9223 B) (Table 5-1) Laboratories also record information on Total Coliforms and Presence/Absence of *E. coli*. While the USEPA's recommended holding time is 8 hours, it is not possible for crews to submit samples to laboratories in that timeframe. For the purposes of the NLA, crews will ship samples to the analysis laboratory as soon as is practicable (either the same day as sample collection or the following day) via overnight courier and the laboratories will begin processing the samples within 6 hours of receipt to minimize exceeding the holding time as much as possible. The minimum detection limit for this analysis is one Most Probable Number (MPN) per 100mL of sample, and the maximum detection limits is up to 2419 MPN per 100mL of sample.

**Table 5-1 Bacteria: analytical methods.**

Storage Requirements	Type	Method
Maintain at 4 C°	Bacteria	Standard Method 9223 B using IDEXX Colilert and Quanti-Tray/2000

Crews will collect 200 ml of surface water in a 290 mL shrink-banded sterile IDEXX bottle (plastic) that will later be separated into two 100 ml aliquots during test preparation to allow for duplicate testing, if necessary. Crews will ship the samples overnight to the laboratory on wet ice maintaining a temperature of 4 °C. The laboratories will analyze an undiluted water sample from the sample<sup>d</sup>. The laboratory technician will add the Colilert® reagent directly to one 100 mL aliquot of undiluted sample and mix it thoroughly to dissolve the reagent. If a laboratory's duplicate is run, the laboratory will follow the same procedure for the second 100 mL aliquot. The technician transfers the sample to QuantiTrays®/2000 (Figure 5.1) and seals the trays using the Quanti-Tray sealer. Samples are incubated at 35.0 ± 0.5° C for 24 hours. Results are reported as MPN/100 mL.



**Figure 5.1 Bacteria (*E.coli*): disposable 97-well tray for use with the Quanti-Tray sealer**

Image from the IDEXX website: <https://www.idexx.com/water/products/quant-tray.html>

<sup>d</sup> Because NLA participants will not know which sites need to be diluted, and with holding time constraints, it will not be possible to run dilutions on the NLA 2017 samples. Analyses that show greater than the 2419 count will be reported as having a result of 2419 with a flag that indicates the value has exceeded the upper limit of quantitation.

## 5.1 Responsibility and Personnel Qualifications

All laboratory personnel are trained in advance in the use of equipment and procedures used during the implementation of this SOP. All personnel are responsible for complying with all of the QA/QC requirements that pertain to this indicator.

## 5.2 Precautions

The analysis involves handling of freshwater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently. The Colilert® reagent is not hazardous although the manufacturer does recommend wearing gloves and safety glasses while using this reagent and washing hands after use.

Interferences - water samples containing humic or other material may be colored. If there is background color, compare inoculated trays to a control tray containing only water (SM, 9223 A).

### 5.2.1 Storage and Stability

Colilert 100 mL format can be stored up to 12 months at 2–30°C.

## 5.3 Equipment/Reagents/Standards

Sample from field crew

Sterile bottles with cap for duplicates

Quanti-Tray Sealer® and rubber inserts

Incubator

Colilert® reagent: Snap packs for 100 ml samples, IDEXX

Quanti-Tray®/2000: Containing 97 wells each, IDEXX.

Squeeze bottles for blank

Sterile pipet for removing excess volume in sample container if needed

UV light

## 5.4 Sample Receipt

Because USEPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps upon receiving a delivery.

1. Report receipt of samples to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** of the manual for contact information).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
  - a. Verify that the sample IDs in the shipment match those recorded on the sample tracking form
  - b. Record the information in Table 3.3-1 for the NARS IM Team, 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. *W*: Sample is warm (>4°), record the temperature in the comment field, and perform the assay
- c. If any sample is damaged or missing, contact the USEPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).
3. Store samples in the refrigerator until sample preparation begins.
  4. Maintain the sample tracking forms with the samples.

**Table 5-2 Bacteria login: required data elements.**

FIELD	FORMAT	DESCRIPTION	
LABORATORY ID	text	Name or abbreviation for laboratory	
DATE RECEIVED	MMDDYY	Date sample was received by laboratory	
SITE ID	text	NLA 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 (>4°)
		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	

## 5.5 Procedure

1. Record all site identification numbers and sample identification numbers being analyzed in the data spreadsheet.
2. Record initial temperature of samples in the data spreadsheet.
3. The laboratory technician then implements the following steps for test preparation and test procedure outlined in **Sections 5.5 and 5.5.2**.

### 5.5.1 Test preparation

1. Turn on Quanti-tray Sealer and warm it to  $35.0 \pm 0.5^{\circ}\text{C}$ . Warm up time is approximately 10 minutes.
2. Shake the water sample bottle 25 times within 7 seconds making sure that the interval between shaking and measuring the test portion is not greater than three minutes.
  - a. If there is at least 1" of headspace, the sample is shaken in the field sample container. Then proceed to step 3.
  - b. If there is insufficient headspace (<1") for proper mixing, do not pour off the excess and discard. Instead, pour the entire sample into a larger sterile container, mix properly and proceed to step 3. Record this on the data spreadsheet.
3. Transfer 100 mL of sample into a sterile bottle with cap and sufficient volume to allow for at least 1" of headspace. For duplicates, the laboratory transfers the second 100 mL into another sterile bottle.

### 5.5.2 Test Procedure (see also manufacturer's instructions for preparation of Quanti-Tray/2000 and use of the Quanti-Tray Sealer)

1. Open a Colilert ampule (reagent) and pour contents into one of the undiluted samples.
2. Re-cap the bottle and shake until reagent is dissolved.
3. Label back of tray with sample ID. If this is a laboratory duplicate or a laboratory reagent blank, make sure the label clearly indicates this.
4. Use one hand to hold open the Quanti-Tray/2000 with the well side of the tray facing your palm.
5. Squeeze upper part of the tray so it bends toward the palm.
6. Gently pull open the tray. Avoid touching the inside of tray or the foil tab.
7. Pour 100 ml of the sample into the tray.
8. Tap the small wells 2-3 times to release bubbles. Failure to release the bubbles may result in the wells filling or sealing improperly.
9. Place the tray with the sample into the rubber insert so that the wells sit within the cutouts.
10. Place the rubber insert on the input shelf of the sealer.
11. Slide the rubber insert with the tray into the sealer.
12. Place the sealed tray/trays into the incubator and incubate at  $35.0 \pm 0.5^{\circ}\text{C}$  for 24 hours.
13. Record the lot number of the reagents and the wells used in the data spreadsheet.

### 5.5.3 Results

1. From the back of the tray, record the sample ID in the data spreadsheet corresponding to the correct NLA site ID. Count the number of small and large positive (yellow) wells and refer to the MPN table to find the most probable number for Total Coliform. Use the color comparator to confirm positive results. Document this number in the data spreadsheet.
2. Use an Ultra Violet lamp to check for fluorescence.
  - a. If no wells fluoresce, the test is negative for E. coli. If wells do fluoresce, the test is positive for E. coli. Record Presence or Absence in the data spreadsheet.

- b. Count the small and large fluorescing wells. Refer to table for most probable number (MPN). Record the results in the data spreadsheet.
3. Calculate precision for laboratory duplicates. The desired precision objective is  $\pm 20\%$  (RPD).

#### Equation 5.1 Precision

$$\text{Precision (as RPD)} = \frac{(A - B) \times 100\%}{(A + B)/2}$$

Where: A = MPN from aliquot A and  
B = MPN from aliquot B

#### 5.5.4 Data Entry

Required data elements that laboratories must provide to the USEPA, are identified in the USEPA's data template, available separately from the USEPA. If the laboratory applies its own QC codes, the data transmittal must define the codes.

### 5.6 Pertinent QA/QC Procedures

Table 5-3 provides a summary of the quality control activities.

#### 5.6.1 Internal QC

1. Initial laboratory demonstration of capability and for each new lot of Quanti-Tray/2000:
  - a. Use an Ultra Violet lamp to check for fluorescence of the media snap packs. Discard the lot if fluoresces.
  - b. Dissolve one packet in 100 ml distilled water. Do not incubate. Check for fluorescence. If it fluoresces, discard the lot.
2. Quanti-Cult Procedure (Validation of performance of Colilert) run once per new lot of Quanti-Tray/2000. See Quanti-Cult instructions from manufacturer.
  - a. Pre-heat incubator to bring temperature up to  $35^{\circ} \pm 0.5^{\circ}\text{C}$ .
  - b. Pre-warm rehydration fluid vials at  $35^{\circ}\text{C}$  for 10 minutes.
  - c. Transfer colorless cap from desiccant vial onto pre-warmed rehydration vial. Discard the blue cap and desiccant vial.
  - d. Place the vial into the foam rack/vial holder (provided with Quanti-Cult kit).
  - e. Invert the foam rack and place in the incubator for 10 minutes.
  - f. Fill four sterile IDEXX 100 ml (or other appropriate, sterilized bottles) bottles with distilled water to the fill line.
  - g. Label three bottles with each bacteria name and one bottle "control".
  - h. Place in incubator until a temperature of  $35^{\circ} \pm 0.5^{\circ}\text{C}$  is reached.
  - i. Remove the rehydration vial from the holder (one at a time). Hold the vial upside down and tap cap gently to mix. Remove the cap and look at the inside surface to ensure that no undissolved black particles are present. Inoculate an additional 10 minutes if present.
  - j. Add entire contents of each appropriate bacteria vial to pre-warmed 100 ml labeled bottles.
  - k. Add the Colilert reagent to sample bottles including the control.
  - l. Place sample bottles in the incubator for 24 hours at  $35^{\circ} \pm 0.5^{\circ}\text{C}$ . Do not place in Quanti-Trays.

The following organism results should be observed:

- Escherichia coli* – Yellow wells, fluorescence
- Klebsiella pneumonia* – Yellow wells, no fluorescence
- Pseudomonas aeruginosa* – Clear wells, no fluorescence
- Method Blank – Clear wells, no fluorescence

3. Sample Batch QC: The analyst runs -
  - a. One laboratory duplicate for every ten samples (laboratory duplicate - two replicates taken from the same collection bottle)
  - b. One (LRB) per sample batch to verify that there is a negative result from 24-28 hours.

### 5.6.2 External QC

1. Analyze 10 provided spiked samples (blind sample) provided by the USEPA. After processing the samples, the laboratory will send the results to the USEPA HQ Laboratory Review Coordinator. The results will be compared to the known concentrations and a determination made. Expected success is correct analysis of 9 of 10 samples with no false negatives.

**Table 5-3 Bacteria: quality control activities for samples.**

Activity	Evaluation	Corrective Action
Demonstrate competency for analyzing <i>E. coli</i> samples to meet the performance measures	Demonstration of competency with <i>E. coli</i> samples in achieving the method detection limits, accuracy, and precision targets.	The USEPA will not approve any laboratory for NLA sample processing if the laboratory cannot demonstrate competency. In other words, the USEPA will select another laboratory that can demonstrate competency for its NLA samples.
Check condition of sample when it arrives.	Sample issues such as cracked container; missing label; sufficient volume for test.	Assign appropriate condition code identified in <b>Table 5-2</b>
Store sample appropriately. While stored at the laboratory, the sample must be kept at a temperature - refrigerated at 4°C.	Check the temperature of the refrigerator per laboratory's standard operating procedures.	Record temperature of sample upon arrival at the laboratory. If at any other time, samples are warmer than required, note temperature and duration of deviation in comment field in <b>Table 5-2</b>  Data analyst will consider temperature deviations in evaluating the data. The analyst will flag the deviations and determine whether the data appear to be affected and/or the data should be excluded from the analyses.
Analyze sample within holding time (8 hour holding time will not be)	The 24-hour test must be completed within 30 hours of receipt (e.g. analysis of this sample must begin	Perform test, but note reason for performing test outside of a 36 hour holding time (assume sample collected by crew at 12:00 PM). The

met, so a 36 hour holding time is the objective for samples)	upon receipt because of holding times.)	USEPA expects that the laboratory will exercise every effort to perform tests before the holding time expires. There will not be sufficient sample for a re-test.
Perform one laboratory reagent blank once at the start of each batch	Control limits cannot exceed the method detection limit of 1 MPN/100 mL).	First, prepare and analyze one additional blank. If the second blank meets the requirement, then no further action is required. If the second blank fails, then determine and correct the problem (e.g., contamination, instrument calibration) before proceeding with any sample analyses keeping in mind that samples must be processed within holding times. Consider alternative options for analyzing samples such as using equipment/laboratory space not involved with the failed blanks. Reestablish statistical control by analyzing three blank samples. Report values of all blanks analyzed.
Compare results of one laboratory duplicate sample	Results must be within the target precision goal of +/-20%.	If both results are below LRL, then conclude that the test has passed. Otherwise, prepare and analyze a split from a different sample in the batch. If the second result is within the target precision goal (see <b>Section 5.5.3</b> ) of the original sample, then report the data and findings for both QC samples. However, if the two results differ by more than the target precision goal, check preparation of split sample; etc. and report evaluation and findings in the case narrative and assign appropriate data code. Consult with the USEPA HQ NLA Laboratory Review Coordinator to determine what if any changes in laboratory protocols are needed.
Maintain the required MDL identified 1 MPN/100 ml	Evaluate for each sample	If MDL could not be achieved, then provide QC code and explanation in the comment field.
Participate in External Quality Control	Evaluate QC samples provided by the External QC Coordinator	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 incorrect or unusual results. 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.
Maintain completeness	Completeness objective is 95% for all parameters.	Contact the USEPA HQ NLA Laboratory Review Coordinator immediately if issues affect laboratory's ability to meet completeness objective.

\*Section 1.2 provides contact information for the USEPA HQ NLA Laboratory Review Coordinator. Laboratories under contract to the USEPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the Laboratory Review Coordinator.

## 6.0 BENTHIC MACROINVERTEBRATE METHODS

This procedure is adapted from the *Wadeable Streams Assessment: Benthic Laboratory Methods* (USEPA, 2004), and is modified to facilitate processing and identification of benthic organisms collected in the littoral zone of lakes and reservoirs.

Benthic macroinvertebrate samples will be preserved in the field with ethyl alcohol (EtOH) and shipped from field crews to a contract batching laboratory. The contract batching laboratory will send the batched samples to the analysis laboratory. Preserved samples will arrive in the analysis laboratory and can be held for several months. If samples are not processed soon after receipt, then periodic evaluation of samples should occur to ensure that sufficient EtOH levels are maintained. Benthic invertebrate analysis laboratories will need to process samples in accordance with the time frame outlined in contractual agreements. Contractual agreements for delivery of data do not supersede indicator holding times.

### 6.1 Responsibility and Personnel Qualifications

This procedure may be used by any person who has received training in identification of freshwater benthic macroinvertebrates, i.e., taxonomy. It is also important that the taxonomist maintains contact with other taxonomists through professional societies and other interactions, and keeps up with the pertinent literature, since systematics and species identifications change over time. A second taxonomist will re-identify a randomly-selected 10% of the samples for QC, as noted below, to quantify enumeration and taxonomic precision, or consistency, as **percent difference in enumeration (PDE)** and **percent taxonomic disagreement (PTD)**, to help target corrective actions, and ultimately to help minimize problems during data analysis. Samples are sent to the laboratory from the field on a regular basis to avoid delays in processing and sample identification.

### 6.2 Precautions

#### 6.2.1 Sorting and Subsampling Precautions

Because it can be difficult to detect the organisms in lake samples (due to inexperience, detritus, etc.), a person who has received instruction from senior biology staff familiar with processing benthic samples must have a QC check performed by qualified personnel (laboratory QC Officers) only. These QC checks will be performed in the pertinent QA and QC Procedures section. The laboratory QC Officers must perform these QC checks immediately following sorting of each grid.

Thoroughly clean all sorting equipment and make sure all equipment is free of organisms prior to sorting the next sample.

#### 6.2.2 Taxonomy Precautions

The USEPA will supply a list of taxa that have been collected from previous iterations of the National Lakes Assessment (provided during laboratory initiation call). The laboratories will use this list as the primary source for taxonomic names to be used in the current NLA sample processing. During the processing of samples, if new taxa are encountered that are not part of the existing NLA taxa list then analysts must provide either a literature citation for this new taxa or its Integrated Taxonomic Information System (ITIS; Web at <http://itis.gov>) number, if available. New taxa will not be excepted unless either of these items are provided.

The analyst must prepare a list of primary and secondary technical literature used in completing the identifications and submit this list to the Project Quality Assurance Manager when samples are returned (see below).

### **6.3 Equipment/Materials**

#### **6.3.1 Sorting and Subsampling Equipment/Materials**

U.S. 35 sieve (500 µm)  
Round buckets  
Standardized gridded screen (370-µm)  
Mesh screen, 30 squares (6 cm<sup>2</sup> each) with white plastic holding tray<sup>1</sup>  
6-cm scoop  
6-cm<sup>2</sup> metal dividing frame ("cookie cutter")  
White plastic or enamel pan (6" x 9") for sorting  
Scissors  
Teaspoon  
India ink pens  
Dropper  
Fine-tipped forceps (watchmaker type, straight and curved)  
Specimen vials with caps or stoppers  
Sample labels for specimen vials  
70-80% denatured ethanol (EtOH)  
Benthic Sample Log-In Form  
Benthic Macroinvertebrate Laboratory Bench Sheet (**APPENDIX B: SAMPLE LABORATORY FORMS**)  
Stereo zoom microscope (6-10X magnification)

#### **6.3.2 Taxonomy Equipment/Materials**

Stereo dissecting microscope with fiberoptics light source (50-60X magnification)  
Compound microscope (10, 40, and 100X objectives, with phase-contrast capability)  
Petri dishes  
Microscope slides (1" x 3" flat, precleaned)  
Cover slips (appropriately sized)  
CMCP-10 (or other appropriate mounting medium)  
India ink pens  
Dropper  
Fine-tipped forceps (watchmaker type, straight and curved)  
Specimen vials with caps or stoppers  
Sample labels for specimen vials

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<sup>1</sup>Some laboratories may choose not to use the gridded screen in a plastic holding tray.

70 - 80% denatured ethanol in plastic wash bottle  
Benthic Macroinvertebrate Taxonomic Bench Sheet  
Hand tally counter

## 6.4 Sample Receipt

Because USEPA 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 to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** of the manual for contact information).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
  - a. Verify that the sample IDs in the shipment match those recorded on the sample tracking form.
  - b. Record the information in **Table 6-1** for the NARS IM Team, 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
  - c. If any sample is damaged or missing, contact the USEPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).
3. Store samples until sample preparation begins.
4. Maintain the sample tracking forms with the samples.

**Table 6-1 Benthic macroinvertebrate login: required data elements**

FIELD	FORMAT	DESCRIPTION		
LAB	text	Name or abbreviation for laboratory		
DATE RECEIVED	MMDDYY	Date sample was received by laboratory		
SITE ID	text	NLA 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.		
		<table border="1"> <thead> <tr> <th>Flag</th> <th>Definition</th> </tr> </thead> <tbody> <tr> <td> </td> <td> </td> </tr> </tbody> </table>	Flag	Definition
Flag	Definition			

		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		Q	Other quality concerns, not identified above
CONDITION COMMENT	text	Comments about the condition of the sample.	

## 6.5 Procedure

### 6.5.1 General

1. Record receipt of samples in the laboratory on the Benthic Sample Log-In form (**APPENDIX B: SAMPLE LABORATORY FORMS**). Assign the appropriate chronological bench number to each sample. Store samples at room temperature until ready for processing.
2. Sample container(s) may arrive with very little alcohol to expedite shipping times and to account for hazardous material handling requirements. Inspect each jar THE SAME DAY THEY ARE RECEIVED and refill them with 70-80% EtOH if necessary. After refilling the sample containers, store them until sorting begins. Check samples periodically to ensure EtOH levels are sufficiently maintained.
3. Use a gridded screen to sort a randomized 500-organism subsample separately from the rest of the sample. Preserve the sorted organisms in one or more specimen vials with 70-80% EtOH.
4. For each sample, document the level of effort, or proportion of sample processed (e.g., number of grids processed), on the Benthic Macroinvertebrate Laboratory Bench Sheet (**APPENDIX B: SAMPLE LABORATORY FORMS**).
5. Record the following information on internal sample labels used for vials of sorted material with India ink pen on cotton rag paper or an acceptable substitute.
  - a. Station Name
  - b. Station Location
  - c. Station Number
  - d. Date Sorted
  - e. Sorter's Initials
  - f. "1 of x" or "2 of x", etc. if the sample is sorted into >1 vial (where x is the total number of vials for the sorted sample)

### 6.5.2 Subsampling

1. Remove the lid from the sample container and remove the internal sample label (save the label—it will need to be returned to the sample container with the archived portion of the sample that does not get processed). Record the sample collection information on a Benthic Macroinvertebrate Laboratory Bench Sheet. Header information required includes both project name and date the sample was collected. Set the bench sheet aside.
2. Carefully decant the alcohol from the sample container by pouring the fluid through a sieve (U.S. 35) into a separate container (the alcohol is saved to preserve the archived portion of the sample that does not get processed). Inspect the mesh of the sieve for any organisms and return any organisms found back to the sample.

3. Transfer the homogenized sample material to the gridded screen portion of the grid (use more than one subsampling device if necessary). Wash the sample thoroughly by running tap water over it to remove any fine material. If there is more than one jar for any particular sample, empty and wash each jar onto the Caton-type grid one at a time, making sure to spread each jar's contents evenly across the tray. Multiple jars from the same sample should all be emptied onto the same Caton grid (or suitable alternative subsampling tray). If the amount of leaf litter or other detrital material exceeds that which fills the tray to the level of the wall panels (it should be spread as evenly as possible), divide it among two or more trays.
4. NOTE: Elutriation of a sample is acceptable for samples with heavy amounts of inorganic substrate (e.g., sample that has 4 or 5 jars total and 2 or 3 with gravel or sand) once it has been delivered to the laboratory, before subsampling has begun on that particular sample. Magdych 1981 describes an inexpensive, easily constructed elutriator. An example of an acceptable elutriation method is as follows:
  - a. Pour alcohol off of sample containers through sieve (at least 500  $\mu\text{m}$ ). Also deposit leaf litter and any other **organic material** (leaves, sticks, algae) onto sieve.
  - b. Depending on amount of **inorganic material** (gravel, sand, silt), pour all or a portion of this material into a rectangular Tupperware/Rubbermaid container and cover with water.
  - c. Circulate (elutriate) sample with water and allow any organisms that might be in the gravel/sand to float to the top of the water and pour the water through the sieve.
  - d. Repeat this until the water runs clear.
  - e. Fill the plastic container (that still has the inorganic material in it) with water one more time and take it to a well-lit, flat surface. Inspect it here under a ring light w/ 3x magnification for any remaining organisms. Have another sorter double check for organisms.
  - f. Once you are certain there are no organisms remaining in the plastic container, wash the water through the sieve and dump the inorganic material into a waste bucket.
  - g. Repeat this process until all of the inorganic material has been elutriated and checked for heavier organisms, such as clams, mussels, or worms.
5. Spread the sample now in the circular sieve over the 30-grid Caton tray.
6. Place the gridded screen into the larger white tray. (Note: Some laboratories may not use the gridded screen and holding tray). Add enough water to spread the sample evenly throughout the grid (the water level should be relatively close to the top of the white tray). Spread the sample material over the bottom of the pan as evenly as possible. Move the sample into the corners of the pan using forceps, spoon, or by hand. Vibrate or shake the pan gently to help spread the sample.
7. Lift the screen out of the white tray to drain. Pour off or siphon excess water from the white tray and set the screen back into the tray. Leave just enough water in the bottom of the tray so that it barely covers the screen once it is returned to the tray to allow the sample to remain moist.
8. Use a random number generator to select at least 10% of the grids (usually 3 grids in a 30-grid tray) to process (select one letter and one number, e.g., A-5, F-2). A minimum of three grids (Canton tray or larger grid size), or 10% of the grids (if a grid of more than 30 squares [ $<6\text{ cm}^2$  each] is used) are sorted from the sample to ensure that the subsample material is representative of the overall sample. Remove all the material from the first grid. If two trays are being used to hold a large sample, remove the material from the same grid on the second pan. Remove the material as follows:
  - a. Place the metal dividing frame or "cookie cutter" over the sample at the approximate location of the grid selected for processing (based on the letters and numbers marked on the sides of the gridded tray). Use a pair of rulers or other straight edges to facilitate lining up the cookie cutter at the intersection if necessary.

- b. Remove the material within the “cookie cutter” using the 6-cm scoop, a teaspoon, forceps, or dropper. Depending on the consistency of what is in the sample, it might be necessary to cut the material along the outside of the “cookie cutter” with scissors or separate it with forceps so that only one grid’s worth of sample material is used. Inspect the screen for any remaining organisms. Use the following rules when dealing with organisms that lie on the line between two grids:
    - i. An organism belongs to the grid containing its head.
    - ii. If it is not possible to determine the location of the head (i.e., for worms), the organism is considered to be in the grid containing most of its body.
    - iii. If the head of an organism lies on the line between two grids, all organisms on the top of a grid and those on the right side of a grid belong in that grid, and are picked with that grid.
  - c. Quarter the grid (if necessary, see **Section 6.5.3, #2**). Place the material from the selected grid(s) into a separate white plastic or enamel pan. Add the necessary amount of water to the pan to facilitate sorting.
9. Set the subsampling device aside in case more grids need to be retrieved later. Cover the sample with aluminum foil to prevent desiccation of the sample and damage to specimens (periodically moisten the sample with water from a spray bottle if the top layer begins to dry). Between each subsampling operation, be careful not to disturb the subsampling device to prevent redistribution of specimens, which could possibly change the probability of selection.

### 6.5.3 Sorting

1. Randomly select at least 10% of the tray or three grids in the case of a Caton tray (assuming 30 grids).
2. If the number of organisms appears to exceed the target number (500 organisms) in the collective three grids, quarter each grid, and randomly select a quarter for initial sorting. Sort the quarter volume of the first grid. Sort the remaining two grids (quartered) in successive order (compositing of the first three grids is not done).
3. If the number of organisms is below the target number, then process another fraction of each grid until the target number of 500 and a maximum of 600 (500+20%) is reached. All organisms from the selected fraction, or grid, must be sorted to minimize bias.
4. If the target is not reached when the three grids are fully processed (including organisms recovered during QC checks), randomly select subsequent grids and pick each to completion until 500+20% organisms is reached. If the target number of organisms is reached within the fraction of the first or second grids, stop sorting for that sample on completion of the sorting of the corresponding fraction (i.e., the third grid quarter would not be processed).
5. If the target level of 500 organisms is not reach within 20 hours of sorting, stop sorting and preserve the remaining unsorted material in 70-80% denatured EtOH, and store for future sorting, if needed.
6. Remove the macroinvertebrates from the detritus with forceps. Sort all samples under a minimum of 6x (maximum of 10x) dissecting microscope. Perform QC checks using the same power microscope. Place picked organisms in an internally-labeled vial (or larger container, if necessary) containing 70-80% denatured EtOH.
7. Keep a rough count of the number of organisms removed and enter the number of organisms found in each grid under the appropriate column on the Benthic Macroinvertebrate Laboratory Bench Sheet. Enter the sorter’s initials in the appropriate column on the bench sheet for each grid sorted.
8. Do not remove or count:

- a. Empty snail or bivalve shells
  - b. Specimens of surface-dwelling or strict water column<sup>2</sup> arthropod taxa (e.g., Collembola, Veliidae, Gerridae, Notonectidae, Corixidae, Culicidae, Cladocera, or Copepoda)
  - c. Incidentally-collected terrestrial taxa.
9. Also, do not count fragments such as legs, antennae, gills, or wings.
  10. For Oligochaeta, attempt to remove and count only whole organisms and fragments that include the head; also, do not count fragments that do not include the head. If a sorter is unsure as to whether a specimen should be counted or not, he or she should place the organism in the sort vial without counting it (the final count is made by the taxonomist).
  11. Once it is picked by the initial sorter, an experienced, certified, laboratory QC Officer must check each sample for missed organisms before another sample is processed. The laboratory QC Officer will count any missed organisms found and place them into the sample vial, or other suitable sample vial. The laboratory QC Officer will note the number of organisms missed on the Benthic Macroinvertebrate Laboratory Bench Sheet, and add that number to the final count of the sample.
  12. If the last grid (or quarter) being processed results in more than 600 organisms (i.e., > 20% above target number), evenly redistribute all of the organisms (without detritus) in a Petri dish (or other small container, i.e., finger bowl, etc.) divided into pie slices (1-8) containing just enough water to cover the sample. Randomly choose slices and count organisms that are wholly contained within the slices. If an organism is lying between two slices, use the criteria in **Section 6.5.2 #8 (B)** to determine which slice it belongs in. Choose slices until you reach the target number (500 +20%). As with picking grids and quarters, you must pick an entire pie slice, even if the sample goes over 500 organisms as long as it remains under 600 total organisms.
  13. Once the QC check of the material in the pan has been completed, remove the material from the pan and place it in a separate container with preservative (70-80% EtOH). Label the container "Sorted Residue," on both internal and external labels ("Sorted Residue" will include material from all grids processed for each sample). Internal sample labels should be made of cotton rag paper or an acceptable substitute, recording the same information as before.
  14. After the laboratory QC Officer completes the QC check, and the target number has been reached, search the entire tray for 5-10 minutes, looking for large/rare organisms (Vinson and Hawkins, 1996). Large/rare is defined as any organism larger than 0.5" long and found in less than one eighth of the tray holding the entire sample. Place any organisms found into a vial labeled "L/R" for "Large/Rare."
  15. Return all material not subsampled (remaining on the grid) to the original container with the preservative. This container will include the original sample labels. Prepare two additional labels "Unsorted Sample Remains" and place one inside the container and attach the other to the outside of the container. Replace the lid and tighten securely. Archive the container until all appropriate QC checks are completed (subsampling and taxonomy). The decision to discard any sample portion should be done only following joint approval of the laboratory QC Officer and the Project Manager.
  16. Record the sorting date each sample was completed near the top right corner of the bench sheet.

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<sup>2</sup>Strict water column taxa are those that do not have at least one life stage that is benthic (i.e., bottom-dwelling).

#### 6.5.4 Taxonomy Procedures

1. The taxonomic target for benthic invertebrates is identified in **Section 6.5.1 #3**
2. Upon receipt of a set of sample vials from the project cooperater or the contractor batch laboratory, remove the sample tracking form from the shipping container, and sign and date it in the "received by" space to verify that the samples were received. Compare all sample numbers on the form with those entered on the labels of samples that actually were in the shipment. If any vials were broken, notify the project facilitator immediately. Maintain the sample tracking form with the samples; it will be needed to return the samples.
3. Empty one sample vial at a time into a small Petri dish. Add 80% denatured EtOH to keep the organisms covered. Remove the internal sample label and complete the top portion of a Benthic Macroinvertebrate Taxonomic Bench Sheet, using the information from the label or that provided by the project facilitator.
4. View the sample under the stereo dissecting microscope and remove similar organisms to other dishes (keep these covered with 80% EtOH). Identify organisms to the correct taxonomic level for the project (usually genus, **Table 6-2**). However, according to the laboratory manager's discretion, a taxonomist can identify any organism finer than the target level if he or she is confident in the identification. Record the identifications on the Benthic Macroinvertebrate Taxonomic Bench Sheet (under taxon). Enter the number of larvae, pupae, and adults of each taxon under those columns on the bench sheet. Use the following steps to compare the final taxa list for each site to that of the provided USEPA NLA taxa list.
  - a. Merge the USEPA provided NLA taxa list with the laboratory electronic bench sheet data by merging the TARGET\_NAME from the NLA taxa list to the TAXON\_NAME from the individual sample data.
  - b. Any taxa in the individual sample data that do not match a name from the NLA taxa list should be checked for the following potential issues. If after this is completed and it is determined that the non-matched taxa is unique then this taxa name can be included, but only after either a literature citation or an ITIS number are provided.
    - i. Abbreviations
    - ii. Extra information identifiers (e.g., sp., spp., , nr., cf., genus 1, w/ hair chaete)
    - iii. Extra character (e.g., "?", "Acentrella ?turbida", blank space)
    - iv. The word "probably" or "prob" (e.g., "Microcylloepus prob. similis")
    - v. Identifying to a lower level than in ITIS (e.g. to species rather than genus)
    - vi. Double names (e.g., Callibaetis callibaetis)
    - vii. Common misspellings
    - viii. Tribes/subfamilies/subgenus sometimes do not appear in ITIS
    - ix. Species with incorrect genus (Hydatopsyche betteni)
    - x. Split level taxonomy (e.g., Cricotopus/Orthocladius)
    - xi. Invalid name (e.g., taxonomic change, synonym; Sphaeriidae vs. Pisiidae)
    - xii. Valid name, in scientific literature, but not in ITIS (e.g., appears in Merritt & Cummins (1996) or Epler (2001), but not listed in ITIS - will not have a TSN)
5. Prepare slide mounts of Chironomidae and Oligochaeta as needed using CMCP-10 (or CMC-9, CMC-10, or other media) and applying a coverslip. View these organisms under the compound microscope to ensure that all necessary diagnostic characters have been observed, according to the taxonomic key or other literature. Record the identifications on the bench sheet as above. Label the slides with the same sample number or log-in number as the alcohol specimens.

6. Prepare a list of primary and secondary technical literature used in completing the identifications. Provide complete citations in bibliographic format, including authors' names, date of publication, title of document, name of journal or publisher, volume and page numbers, or ISBN number, as appropriate. These will be kept on file with the project QC officer.
7. If damaged organisms can be identified, they are counted ONLY if:
  - a. the fragment includes the head, and, in the case of arthropods, the thorax; the analysts should match the detached head and thorax parts to ensure that double counting of individuals does not occur.
  - b. oligochaetes, heads with a sufficient number of segments;
  - c. the mollusk shell (bivalve or gastropod) is occupied by a specimen;
  - d. the specimen is the sole representative of a taxon in the sample.
8. If early instar or juvenile specimens can be identified, they are counted as separate taxon.
9. Add the number of organisms from each developmental stage and enter the total on the bench sheet.
10. Complete the bench sheet by entering the totals for each developmental stage and the total number of each taxon in the cells at the bottom of the sheet. Cross-check to be sure the totals were summed correctly. Make a copy of the bench sheet for the project file.
11. Create a reference collection with at least one specimen from each genus (or lowest taxonomic level identified). The taxonomist must choose an appropriate specimen(s) to represent each taxon in the master taxa list. The specimen(s) must be removed from the sample and placed in the reference collection. Circle slide-mounted specimens with a grease pencil (or other appropriate mark) to indicate those which belong to the reference collection. For all slides containing reference and non-reference specimens, be sure to place a label in the sample container that does not contain the reference collection. Each laboratory must maintain a master list of taxa recorded. The contract lead will coordinate any necessary inter-laboratory communication and produce and integrated master taxa list for the project.
12. Carefully return the rest of the organisms to the original sample vial, fill with 70-80% denatured EtOH, and cap tightly.
13. Re-package the samples and slide-mounted specimens carefully, and sign and date the sample tracking form in the next "relinquished by" space. The samples must be shipped, properly packed in a box, by overnight carrier to the Project Facilitator, and receipt must be confirmed by the person doing the shipping. Each taxonomist must retain a full set of bench sheet copies and ship the original bench sheets to the contract lead. Ship samples and bench sheets separately.

#### **6.5.4.1 Taxonomic Level of Effort**

This is the Standard Taxonomic Effort list for benthic macroinvertebrates (**Table 6-2**). It represents the minimum level needed for mature and well preserved specimens. The lowest targeted taxonomic level will be genus. Due to taxonomic limitations, some groups cannot be identified to the genus level and therefore should be taken to the level specified below. For all taxonomic groups, if the level can easily go lower, for example monotypic genera, or if only one genus or species is known to occur in a certain geographic area, then these specimens should be identified at the lowest possible taxonomic level (e.g., Ephemerellidae *Drunella doddsi*). If the minimum taxonomic level cannot be achieved due to immature, damaged, or pupal specimens this should be noted in the data file with a "flag" variable (e.g., IM = y, DD = y, PP = y).

**Table 6-2 Required level of taxonomic identification for benthic macroinvertebrates.**

Phylum	Class		Required Taxonomic Identification	Notes
<b>ANNELIDA</b>				
	Branchiobdellida		Family	
	Hirudinea		Genus	
	Oligochaeta		Genus	
	Polychaeta		Family	
<b>ARTHROPODA</b>				
	Arachnoidea			
		Acari	Genus	
	Insecta			
		Coleoptera	Genus	
		Diptera	<b>Except in the following cases:</b>	
			Chironomidae	Genus this may not be possible for some groups, which should be identified to at least tribe or subfamily
			Dolichopodidae	Family
			Phoridae	Family
			Scathophagidae	Family
			Syrphidae	Family
		Ephemeroptera	Genus	
		Hemiptera	Genus	
		Lepidoptera	Genus	
		Megaloptera	Genus	
		Odonata	Genus	
		Plecoptera	Genus	
		Trichoptera	Genus	
	Malacostraca		Genus	
		Amphipoda	Genus	
		Decapoda	Genus	
		Isopoda	Genus	
		Mysidacea	Genus	
<b>COELENTERATA</b>				
<b>MOLLUSCA</b>				
	Bivalvia		Genus	
	Gastropoda		<b>Except in the following case:</b>	
			Hydrobiidae	Family
<b>NEMERTEA</b>				
			Genus	

## 6.6 Pertinent QA/QC Procedures

### 6.6.1 Sorting and Subsampling QC

1. A QC Analyst will use 6-10X microscopes to check all sorted grids from the first five samples processed by a sorter to ensure that each meets the acceptable criteria for percent sorting efficiency (PSE), which is 90%. This will not only apply to inexperienced sorters, but also to those initially deemed as “experienced.” Qualification will only occur when sorters achieve PSE ≥ 90% for five samples consecutively.
2. The laboratory QC Officer will calculate PSE for each sample as follows:

**Equation 6.1 Percent sorting efficiency (PSE).**

$$PSE = \frac{A}{A + B} \times 100$$

where A = number of organisms found by the primary sorter, and B = number of recoveries (organisms missed by the primary sort and found during the QC check).

3. If the sorting efficiency for each of these five consecutive samples is ≥ 90% for a particular individual, this individual is considered “experienced” and can serve as a laboratory QC Officer. In the event that an individual fails to achieve ≥ 90% sorting efficiency, he or she will be required to sort an additional five samples and continue to have their sorting efficiency monitored. However, if he or she shows marked improvement in sorting efficiency prior to completion of the next five samples, achieving ≥ 90% sorting efficiency, the laboratory QA Officer may, at his/her discretion, consider this individual to be “experienced”. Do not calculate PSE for samples processed by more than one individual.
4. After individuals qualify, 10% (1 out of 10, randomly selected) of their samples will be checked.
5. If an “experienced” individual fails to maintain a ≥ 90% PSE as determined by QC checks, the laboratory QC Officer will perform QC checks on every grid of five consecutive samples until a ≥ 90% sorting efficiency is achieved on all five. During this time, that individual will not be able to perform QC checks.

### 6.6.2 Taxonomic QC

#### 6.6.2.1 Internal Taxonomic QC

As directed by the Indicator QC Coordinator, an in-house QC Analyst will conduct an internal 10% re-identification of all samples identified by that laboratory to ensure that each meets the acceptable criteria for percent identification efficiency which is 90%.

If the individual fails to maintain a ≥ 90% identification as determined by QC checks, previous samples will be re-counted and identified.

#### 6.6.2.2 External Taxonomic QC

1. Upon receipt of the data, the Indicator QC Coordinator for macroinvertebrates will randomly select 10% of the samples. The Indicator QC Coordinator will then have the original laboratory send those samples to a QC taxonomist (another experienced taxonomist who did not participate in the original identifications). The original laboratory will complete a sample tracking form and send with the samples.
2. The QC taxonomist will perform whole-sample re-identifications, taking care to ensure inclusion of all slide-mounted specimens and completing another copy of the Benthic Macroinvertebrate Taxonomic Bench Sheet for each sample. Label each bench sheet with the term “QC Re-ID.” As each bench sheet is completed, fax it to the Project Facilitator.

- The Indicator QC Coordinator will compare the taxonomic results (counts AND identifications) generated by the primary and QC taxonomists for each sample and calculate percent difference in enumeration (PDE) and percent taxonomic disagreement (PTD) as measures of taxonomic precision (Stribling et al. 2003) as follows:

**Equation 6.2 Percent difference in enumeration (PDE).**

$$PDE = \frac{|n_1 - n_2|}{n_1 + n_2} \times 100$$

where  $n_1$  is the number of specimens counted in a sample by the first taxonomist and  $n_2$  is the number of specimens counted by the QC taxonomist.

**Equation 6.3 Percent taxonomic disagreement (PTD).**

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

where  $comp_{pos}$  is the number of agreements (positive comparisons) and  $N$  is the total number of specimens in the larger of the two counts.

- The recommendation for PDE is 5% or less.
- A PTD of 15% or less is recommended for taxonomic difference (overall mean < 15% is acceptable). Individual samples exceeding 15% are examined for taxonomic areas of substantial disagreement, and the reasons for disagreement investigated. A reconciliation call between the primary and secondary taxonomist will facilitate this discussion. Results greater than this value is investigated and logged for indication of error patterns or trends.
- Corrective actions include determining problem areas (taxa) and consistent disagreements and addressing problems through taxonomist interactions. These actions help to rectify disagreements resulting from identification to a specific taxonomic level.

**6.6.2.3 Taxonomic QC Review & Reconciliation**

The Indicator QC Coordinator prepares a report or technical memorandum to quantify aspects of taxonomic precision, assess data acceptability, highlight taxonomic problem areas, and provide recommendations for improving precision. This report is submitted to the HQ Project Management Team, with copies sent to the primary and QC taxonomists. Another copy is maintained in the project file. Significant differences may result in the re-identification of samples by the primary taxonomist and a second QC check by the secondary taxonomist.

All samples are stored at the laboratory until the Project Lead notifies the laboratory regarding disposition.

**Table 6-3 Laboratory quality control: benthic indicator.**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>SAMPLE PROCESSING (PICK AND SORT)</b>			
Sample residuals examined by different analyst within laboratory	10% of all samples completed per analyst	Efficiency of picking ≥ 90%	If < 90%, examine all residuals of samples by that analyst and retrain analyst

<b>IDENTIFICATION</b>			
<b>Sorted samples re-identified by different analyst within laboratory</b>	10% of all samples	Accuracy of contractor laboratory picking and identification $\geq 90\%$	If picking accuracy $< 90\%$ , all samples in batch will be reanalyzed by contractor
<b>Independent identification by outside taxonomist</b>	All uncertain taxa	Uncertain identifications to be confirmed by expert in particular taxa	Record both tentative and independent IDs
<b>Use standard taxonomic references</b>	For all identifications	All keys and references used must be on bibliography prepared by another laboratory	If other references desired, obtain permission to use from Project Facilitator
<b>Prepare reference collection</b>	Each new taxon per laboratory	Complete reference collection to be maintained by each individual laboratory	Benthic Laboratory Manager periodically reviews data and reference collection to ensure reference collection is complete and identifications are accurate
<b>External QC</b>	10% of all samples completed per laboratory	PDE $\leq 5\%$ PTD $\geq 85\%$	If PDE $> 5\%$ , implement recommended corrective actions. If PTD $< 85\%$ , implement recommended corrective actions.
<b>DATA VALIDATION</b>			
<b>Taxonomic "reasonable-ness" checks</b>	All data sheets	Genera known to occur in given lakes or geographic area	Second or third identification by expert in that taxon

## 7.0 PHYTOPLANKTON METHODS

This method is adapted from protocols used for the U.S. Geological Survey National Water Quality Assessment program (Charles et al. 2003) to identify and enumerate taxa in phytoplankton samples. The method involves microscopic examination of preserved phytoplankton samples from integrated samples collected from the euphotic zone of the water column.

Phytoplankton samples will be preserved in the field with Lugol's solution and shipped from field crews to a contract batching laboratory. The contract batching laboratory will send the batched samples to the analysis laboratory. Preserved samples will arrive in the analysis laboratory and can be held for several months. Phytoplankton analysis laboratories will need to process samples in accordance with the time frame outlined in contractual agreements. Contractual agreements for delivery of data do not supersede indicator holding times.

### 7.1 Responsibility and Personnel Qualifications

This procedure may be used by any person who has received training in processing and/or identification of phytoplankton samples. It is important that all taxonomists maintain contact with other taxonomists through professional societies and other interactions and keep abreast with the pertinent literature, because taxonomic groupings and nomenclatural basis for species identifications are updated frequently. A second taxonomist will re-identify a randomly-selected 10% of the samples for QC, as noted below, to quantify taxonomic precision, or consistency, as **percent difference (PD)**, to help target corrective actions, and ultimately to help minimize problems during data analysis. Samples are sent to the laboratory from the field on a regular basis to avoid delays in processing and sample identification.

### 7.2 Precautions

Wear appropriate clothing for safety precautions, such as nitrile gloves, rubber apron, long pants, etc.

### 7.3 Equipment/Materials

- Compound microscope (with 10, 40, 100X objectives with 10 - 15X ocular, and epifluorescence capability)
- Utermöhl sedimentation chamber
- Pasteur pipette
- Volumetric cylinder
- Bench sheet
- Phytoplankton Sample Log-In Form
- Phytoplankton Laboratory Sheet
- Labels

### 7.4 Sample Receipt

Because USEPA 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 to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** of the manual for contact information).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
  - a. Verify that the sample IDs in the shipment match those recorded on the sample tracking form.
  - b. Record the information in **Table 7-1** for the NARS IM Team, 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
  - c. If any sample is damaged or missing, contact the USEPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).
3. Store samples until sample preparation begins.
4. Maintain the sample tracking forms with the samples.

**Table 7-1 Phytoplankton login: required data elements.**

FIELD	FORMAT	DESCRIPTION	
LAB	text	Name or abbreviation for laboratory	
DATE RECEIVED	MMDDYY	Date sample was received by laboratory	
SITE ID	text	NLA 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
		Q	Other quality concerns, not identified above
CONDITION COMMENT	text	Comments about the condition of the sample.	

## 7.5 Procedure

### 7.5.1 Prepare Utermöhl Sedimentation Chamber

1. Use a light amount of vacuum grease to attach a cover glass to the bottom of an Utermöhl sedimentation chamber. It is critical that the cover glass be clean and grease free.
  - For tubular varieties of settling chambers, seal a cover glass to the threaded end of the tube and screw the tube into the base assembly.
  - For a plate chamber type of settling chamber, attach the cover glass on the bottom of the base, lock it into place with the metal ring and seal the cylinder on top of the base using a light amount of vacuum grease.
2. Homogenize the concentrated samples by repeatedly inverting the sample bottle. Place a 10-ml aliquot of the sample into the assembled settling chamber. Let the sample settle for at least 8 hours.
3. For the plate chamber type of Utermöhl chamber, drain the volumetric cylinder by sliding over the drainage hole. Slide the cover plate over the chamber without allowing air bubbles to form. Analysis should proceed within a few hours of removing the cylinder.

### 7.5.2 Choose Count Method

#### 7.5.2.1 Determine random fields

1. Using a high oil microscope objective (10-15X objective, 100-1500X total system magnification), identify and enumerate algae in selected, random fields. Enumerate between 8 and 100 fields from each Utermöhl chamber. If necessary, use a second chamber.
2. Choose a random starting place in the upper left-hand quadrant of the counting chamber and approximate the number of fields that must be analyzed (400 natural units [Section 7.5.3] need to be counted with a minimum of 8 and maximum of 100 random fields).
3. Develop a pattern that allows for equal probability of landing in any area of the cell or chamber with the exception of the edges and the center. A maximum pattern with 100 fields is made by having an 8x8 grid, and then subtracting 3 or 4 fields in either direction of the center.

#### 7.5.2.2 Determine transects

1. Using a high oil microscope objective (10-15x objective, 100-1500x total system magnification) with a calibrated stage, identify and enumerate algae along transects, either horizontally or vertically across the Utermöhl plate chamber.
2. Without looking into the microscope, choose a location near the left edge in the upper third of the chamber (if vertical transects are analyzed, choose a location near the top edge in the left third of the chamber). Make a transect by moving only the horizontal stage control (or vertical control for vertical transects) a measured distance.
3. Develop a pattern for the transects that will avoid the center and edges of the chamber. A second Utermöhl chamber can be used, if necessary (400 natural units need to be counted with a minimum of one complete transect).

### 7.5.3 Identify and Enumerate 400 Natural Algal Units

1. Species-level resolution is the taxonomic requirement for phytoplankton which likely means using a magnification of 1000X or higher.
2. Using the pattern developed above, move the microscope stage to a new position in the pattern. Make all movements of the microscope stage without looking through the objectives.

3. Identify and enumerate all algal forms in the field of view: enumerate algal forms using natural counting units. Natural counting units are defined as one for each colony, filament, diatom cell (regardless if colonial or filamentous) or unicell. With the exception of diatoms, identify algal forms to species. Develop a method of selecting taxa that are only partially in view. For example, only count taxa that are partially in the field of view if they are on the left side. If they are on the right do not count.
4. Count only “living” diatoms at the time of collection. If there is any protoplast material in the frustule, the diatom is considered to have been living when collected.
5. Differentiate diatoms to the lowest practical taxonomic level. This will usually be genus, but use of categories such as naviculoid, cymbelloid, centric, nitzschoid is appropriate.
6. Count the number of algal cells comprising each multicellular counting unit.
7. Tabulate the data on a bench sheet **APPENDIX B: SAMPLE LABORATORY FORMS**, mechanical or electronic tabulator.
8. Repeat steps 1 - 4 until 400 natural algal units have been enumerated. Again, count only “living” diatoms as part of the required 400 natural algal units.
9. Add and record the tallies of each taxon on the bench sheet. Record the number of cells for multicellular counting units in parentheses beside the tally of natural counting units.
10. Record the number of fields or the total transect length for the area that was observed.

#### 7.5.4 Identify and Enumerate Larger, Rarer Taxa

There is an additional procedure that can be used for samples with low concentrations (less than five natural counting units) of large cells or colonies (maximum dimension greater than 100  $\mu\text{m}$ ).

1. Using a low-power objective (10-15X), scan 20 fields or 4 transects. Count the larger, rarer taxa (as defined above).
2. Enumerate as natural units and estimate the number of cells in each. Record the counts of each of the taxa on the bench sheets, noting the scan area (i.e., total area for the 20 microscope fields or 4 transects). Multiply the number of larger, rarer taxa by the ratio of the total area scanned in the regular count to the area scanned in this count.
3. Record that number as the total count for that taxon.

#### 7.5.5 Measure Cell Biovolumes

1. For each group of samples, measure the dimensions of the taxa that contribute most to sample biovolume. Cell biovolumes of all identified taxa will be quantified on a per milliliter basis. Use formulae for solid geometric shapes that most closely match the cell shape (Hillebrand et al., 1999) to estimate biovolume. Base biovolume calculations on measurements of 10 organisms per taxon for each sample where possible.
2. Biovolumes for each abundant taxon (i.e., occurring in more than 5% in any one sample) should be based on measurements of 10 cells or more
3. Biovolumes for each common taxon (i.e., occurring 2 – 5% in any one sample) should be based on measurements of one or more cells.
4. Biovolumes for each rare taxon (i.e., occurring in 0.1 – 2% in any one sample) should be based on measurements from literature descriptions of taxa, previous measurements of the taxon, or measurements of one or more cells.
5. For taxa with substantial size variation (e.g., diatoms), designate size classes based on sample quality to determine average cell size (biovolume). For each taxon, measure 10 cells from each size class (assuming that sufficient numbers are available). Use mean biovolumes within each size class to calculate the total biovolume contributed by the taxon to its representative sample (Burkholder and Wetzel, 1989).

## 7.6 Calculation and Reporting

1. The calculation of phytoplankton abundance depends on the apparatus used during analysis. Biovolume values are determined by multiplying the abundance (cells/mL) by the average biovolume of each cell ( $\mu\text{m}^3$ ). Phytoplankton abundance (cells/mL) is calculated as follows:

**Equation 7.1 Phytoplankton abundance.**

$$\text{cells/mL} = \left( \frac{\text{count} \times \text{chamber} \times 1000\text{mL}}{\text{numfields} \times \text{field} \times \text{mlsettled}} \right) / 1000$$

where *count* = number of cells counted, *chamber* = chamber area (in  $\text{mm}^2$ ), *numfields* = number of microscope fields, *field* = microscope field area (in  $\text{mm}^2$ ), and *ml settled* = number of ml settled in Utermöhl chamber.

2. Prepare a spreadsheet file containing the count data, using the columns (fields) as shown in the **(APPENDIX B: SAMPLE LABORATORY FORMS: Phytoplankton Measurement Data Sheet)**. Submit the file electronically to the USEPA.

## 7.7 Pertinent QA/QC Procedures

**Table 7-2** provides a summary of quality control procedures for the phytoplankton indicator.

### 7.7.1 Internal Taxonomic QC

An in-house QC Analyst will randomly select 5 of the samples counted and identified by individual taxonomists to ensure that each meets the acceptable criteria for percent identification efficiency which is 90%.

If the individual fails to maintain a  $\geq 90\%$  identification as determined by QC checks, previous samples will be re-counted and identified.

### 7.7.2 External Taxonomic QC

EPA may implement an external taxonomic QC review process for zooplankton. If EPA implements an external QC process, upon receipt of the data after initial identification, the Indicator QC Coordinator for phytoplankton randomly selects 10% of the samples for external QC analysis. The Indicator QC Coordinator will direct the original laboratory to send those samples to a QC taxonomist, a second experienced taxonomist who did not participate in the original identifications. The original laboratory will complete a sample tracking form and send it with the samples.

#### 7.7.2.1 Plankton Re-identification

Duplicate processing (duplicate the processing steps presented in **Section 7.5.1 – 7.5.5**).

The remaining concentrated sample will be sent to the QC taxonomist.

1. Using the same volume as the original Utermöhl chamber, prepare a duplicate Utermöhl chamber cell and enumerate 400 natural algal units. Complete another copy of the Taxonomic Bench Sheet for each sample. Label each bench sheet with the term "QC Dup-ID." As each bench sheet is completed, the laboratory sends it (through email or fax) to the Indicator QC Coordinator.
2. The Indicator QC Coordinator compares the taxonomic results generated by the primary and QC taxonomists for each sample and calculate percent difference using:

**Equation 7.2 Percent difference.**

$$PctDiff = 100 - \sum \min(a,b)$$

where *a* and *b* are the relative proportions recorded for a given taxon by the primary taxonomist (a) and the QC taxonomist (b).

3. Values will be a combination of subsampling error and taxonomic error; the MQO is that the two counts will have a percent difference of ≤ 50.
4. If it appears that high percent difference for soft-bodied phytoplankton are due to subsampling inconsistency, then determine and implement appropriate corrective actions working with the Indicator QC Coordinator. In addition, disagreements resulting from identification to a specific taxonomic level, creating the possibility to double-count “unique” or “distinct” taxa shall be rectified through corrective actions working with the Indicator QC Coordinator.

**7.7.3 Taxonomic QC Review & Reconciliation**

The Indicator QC Coordinator prepares a report or technical memorandum to quantify aspects of taxonomic precision, assess data acceptability, highlight taxonomic problem areas, and provide recommendations for improving precision. This report is submitted to the HQ Project Management Team, with copies sent to the primary and QC taxonomists. Another copy is maintained in the project file. Significant differences may result in the re-identification of samples by the primary taxonomist and a second QC check by the secondary taxonomist.

All samples are stored at the laboratory until the Project Lead notifies the laboratory regarding disposition.

**Table 7-2 Laboratory quality control: phytoplankton indicator.**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>IDENTIFICATION</b>			
<b>Independent identification by outside taxonomist</b>	All uncertain taxa	Uncertain identifications to be confirmed by expert in particular taxon	Record both tentative and independent IDs
<b>Use standard taxonomic references</b>	For all identifications	All keys and references used must be on bibliography prepared by another laboratory	If other references desired, obtain permission to use from Project Facilitator
<b>Prepare reference collection</b>	Each new taxon per laboratory	Complete reference collection to be maintained by each individual laboratory	Laboratory Manager periodically reviews data and reference collection to ensure reference collection is complete and identifications are accurate
<b>External QC</b>	10% of all samples completed per laboratory	Efficiency (PD) ≥ 50%	If PD < 50%, implement recommended corrective actions
<b>DATA VALIDATION</b>			
<b>Taxonomic "reasonable-ness" checks</b>	All data sheets	Genera known to occur in given lakes or geographic area	Second or third identification by expert in that taxon

## 8.0 SEDIMENT CONTAMINANTS, GRAIN SIZE, AND TOC

This method describes the analysis requirements for sediment samples. The purpose is to determine concentrations of contaminants, grain size, and total organic carbon (TOC) in sediment samples collected in the National Lakes Assessment (NLA) 2017 and related studies. The laboratory shall perform analysis to determine the moisture content, concentrations of metals, mercury, pesticides, PAHs and PCBs found in lake sediments.

At each sampling site, the FOM instructs the crews to collect sediment samples. The field crew then ships the samples on wet ice to either its own state laboratory or the USEPA's batching laboratory. Once the samples arrive, the laboratory will freeze the samples for the contaminant analyses and TOC, and will refrigerate the grain size samples.

This chapter describes the contaminant, grain size, and TOC determination of sediment samples collected for the USEPA's NLA 2017. As described in **Section 8.5**, unless otherwise contractually bound by other requirements, the laboratory may choose to use any method that meets the USEPA's specifications for contaminant and grain size measurements.

### 8.1 Definitions and Required Resources (Personnel, Laboratories, and Equipment)

This section provides definitions and required resources for using the analytical procedures.

#### 8.1.1 Definitions

The analytical procedures use the following terms:

**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."

**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.

**NARS Information Management System (NARS IM):** The IM system established to support all surveys, including NLA, in the NARS program. The IM system is used to track the samples from field collection to the laboratory.

**Percent Recovery:** Recovery is measured by comparing the concentrations of a sample split into two parts; where 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:

**Equation 8.1 Percent recovery**

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

**Relative Percent Difference (RPD):** Relative percent difference compares the matrix spike (S) and the matrix spike duplicate (D) using the following equation:

### Equation 8.2 Relative percent difference

$$RPD = \frac{|S - D|}{(S + D)/2} \times 100$$

**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.

**TOC:** Total Organic Carbon

**TOCOR:** Task Order Contracting Officer's Representative is the USEPA's contact person for laboratories under contract to the USEPA.

## 8.1.2 Personnel

The analytical procedures refer to the following personnel:

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

**External QC Coordinator** is an USEPA 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 the NLA. The QC contractor is responsible for complying with instructions from the External QC Coordinator; coordinating and paying for shipments of the PT samples to participating laboratories; and preparing brief summary reports.

## 8.2 Precautions

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. laboratory 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.

### 8.3 Equipment/Materials

The analytical methods, selected by the laboratory, specify the required equipment.

### 8.4 Sample Receipt

Because the USEPA initiates tracking procedures designed to recover any missing shipments, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery. The laboratory must inspect the samples promptly upon receipt. As samples arrive, the laboratory must:

1. Report receipt of samples to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** for contact information).
2. Check that each shipping container has arrived undamaged. Check the temperature of one of the samples in the cooler using either a thermometer that reads from 21 °C (i.e., room temperature) down to -20 °C or lower (i.e., the expected temperature of frozen samples), or an infra-red (IR) temperature “gun” and record the reading.
3. Record the condition and temperature of the sample on the NARS IM sample tracking spreadsheet using the codes in **Table 8-1**.
4. Verify that all required data elements, per **Table 8-1**, have been recorded.
5. Transfer the samples to the freezer or refrigerator for long-term storage. Except during the processing and analysis stages, the contaminant samples and TOC must be stored frozen to less than or equal -20 °C and the grain size samples must be stored in the refrigerator.
6. Notify the USEPA immediately about any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.
7. Maintain the sample tracking forms with the samples.

**Table 8-1 Sediment chemistry, grain size, and TOC login: required data elements.**

Variable	Type	Description	
SITE_ID	Character	Site identification code	
VISIT_NO	Numeric	Sequential visits to site (1 or 2)	
SAMPLE_ID	Character	Sample number	
DATE_COLLECT	MMDDYY	Date that the field crew collected the sample	
ANALYSIS_TYPE	Character	Contaminant, TOC, or GRAIN SIZE	
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
		VT	Volume not sufficient for testing

Variable	Type	Description	
		VR	Volume not sufficient for a retest, if required
		Q	Other quality concerns, not identified above
COND_COMMENT	Character	Explanation for Q FLAG (if needed)	

## 8.5 Laboratory Analysis: Requirements

The laboratory shall perform analyses of the sediment samples to determine the moisture content, grain size, and concentrations of TOC, metals, mercury, pesticides, PAHs, and PCBs.

**Table 8-2** identifies the storage requirements. Laboratories may choose to use any analysis method, including those in **Table 8-2**, which measures the parameters to the levels of the method detection limits identified in **Table 8-3**. In addition, the contaminant analysis method must meet the precision and accuracy targets of 30% and 20%, respectively. For each batch of contaminant samples, precision is assessed using the RPD between the matrix spike (MS) and the matrix spike duplicate (MSD); and accuracy by the average percent recovery (%Rs) between the matrix spike and matrix spike duplicate. **Section 8.1.1** provides the equations used to calculate the RPD and %Rs. The precision and accuracy targets for each batch of TOC are both 10% and determined by the RPD of one sample and its duplicate (for precision) and the analysis of Certified Reference Material (CRM; for accuracy). The grain size target precision is 10% as determined using a Laboratory Control Sample (LCS) (accuracy is not applicable).

**Table 8-2 Sediment chemistry, grain size, and TOC: storage requirements and analytical methods.**

Storage Requirements	Type	Methods that Meet the QA/QC Requirements (any method that meets the QA/QC requirements is acceptable)
Freeze samples with maximum of -20° C	Metals (except Mercury)	Extraction: USEPA Method 3051A Analysis: USEPA Method 6020A <sup>e</sup>
	Mercury	USEPA Method 245.7 <sup>f</sup>
	PCBs, Pesticides, PAHs	Extraction: USEPA Method 3540C Analysis: USEPA Method 8270D <sup>g</sup>

<sup>e</sup> For example, see:

- Method 3051A “Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, And Oils” retrieved April 28, 2017 from <https://www.epa.gov/hw-sw846/sw-846-test-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-soils-and-oils>; and
- Method 6020A “Inductively Coupled Plasma-Mass Spectrometry” retrieved April 28, 2017 from <https://www.epa.gov/sites/production/files/2015-07/documents/epa-6020a.pdf>

<sup>f</sup> For example, see Method 245.7 “Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0” (EPA-821-R-05-001, February 2005), retrieved June 27, 2014 from [http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007\\_07\\_10\\_methods\\_method\\_245\\_7.pdf](http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_245_7.pdf).

<sup>g</sup> For example, see:

	TOC	USEPA Method 9060 <sup>e</sup>
Refrigerate at 4° C (do not freeze)	Grain Size	Any method that reports the determination as %silt and meets QA/QC requirements

**Table 8-3 Sediment chemistry, grain size, and TOC: required parameters.**

Type	UNITS	Parameter	CAS Number	PCB Number (where applicable)	MDL Target
	% sand % silt % clay	Grain Size	not applicable		0.05%
	mg/kg and %	Total Organic Carbon (TOC)	not applicable		0.01%
METAL	dry weight µg/g (ppm)	Aluminum	7429-90-5		1500
		Antimony	7440-36-0		0.2
		Arsenic	7440-38-2		1.5
		Cadmium	7440-43-9		0.05
		Chromium	7440-47-3		5.0
		Copper	7440-50-8		5.0
		Iron	7439-89-6		500
		Lead	7439-92-1		1.0
		Manganese	7439-96-5		1.0
		Mercury	7439-97-6		0.01
		Nickel	7440-02-0		1.0
		Selenium	7782-49-2		0.1
		Silver	7440-22-4		0.3
		Tin	7440-31-5		0.1
Vanadium	7440-62-2		1.0		
Zinc	7440-66-6		2.0		
PCB	dry weight ng/g (ppb)	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	2051-24-3	209	1.0
		2,4'-Dichlorobiphenyl	34883-43-7	8	1.0
		2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170	1.0

- Method 3540C “Soxhlet Extraction” retrieved April 28, 2017 from <https://www.epa.gov/sites/production/files/2015-12/documents/3540c.pdf> ; and
- Method 8270D “Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)” retrieved April 28, 2017 from <https://www.epa.gov/sites/production/files/2015-07/documents/epa-8270d.pdf> .

SEDIMENT CONTAMINANTS, GRAIN SIZE, AND TOC

		2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187	1.0
		2,2',3,4',5,5',6-Heptachlorobiphenyl	35065-29-3	180	1.0
		2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	128	1.0
		2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138	1.0
		2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153	1.0
		2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206	1.0
		2,2',3,3',4,4',5,6-Octachlorobiphenyl	52663-78-2	195	1.0
		2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	105	1.0
		2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101	1.0
		2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	118	1.0
		2,3,3',4,6'-Pentachlorobiphenyl	38380-03-9	110	1.0
		3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	126	1.0
		2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44	1.0
		3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	77	1.0
		2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52	1.0
		2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66	1.0
		2,2',5-Trichlorobiphenyl	37680-65-2	18	1.0
		2,4,4'-Trichlorobiphenyl	7012-37-5	28	1.0
PEST	dry weight ng/g (ppb)	2,4'-DDD	53-19-0		1.0
		2,4'-DDE	3424-82-6		1.0
		2,4'-DDT	789-02-6		1.0
		4,4'-DDD	72-54-8		1.0
		4,4'-DDE	72-55-9		1.0
		4,4'-DDT	50-29-3		1.0
		Aldrin	309-00-2		1.0
		Alpha-BHC	319-84-6		1.0
		Beta-BHC	319-85-7		1.0
		Delta-BHC	319-86-8		1.0
		Alpha-Chlordane	5103-71-9		1.0
		Gamma-Chlordane	5566-34-7		1.0
		Dieldrin	60-57-1		1.0
		Endosulfan I	959-98-8		1.0
		Endosulfan II	33213-65-9		1.0
		Endosulfan Sulfate	1031-07-8		1.0
		Endrin	72-20-8		1.0
		Endrin Aldehyde	7421-93-4		1.0

		Endrin Ketone	53494-70-5		1.0
		Heptachlor	76-44-8		1.0
		Heptachlor Epoxide	1024-57-3		1.0
		Hexachlorobenzene	118-74-1		1.0
		Lindane	58-89-9		1.0
		Mirex	2385-85-5		1.0
		Cis-Nonachlor	5103-73-1		1.0
		Oxychlorane	26880-48-8		1.0
		Trans-Nonachlor	39765-80-5		1.0
PAHs	dry weight ng/g (ppb)	Acenaphthene	83-32-9		1
		Acenaphthylene	208-96-8		1
		Anthracene	120-12-7		1
		Benz(a)anthracene	200-280-6		1
		Benzo(b)fluoranthene	205-99-2		1
		Benzo(k)fluoranthene	207-08-9		1
		Benzo(g,h,i)perylene	191-24-27-2		1
		Benzo(a)pyrene	50-32-8		1
		Benzo(e)pyrene	192-9		1
		Biphenyl	92-54-4		1
		Chrysene	218-01-9		1
		Dibenz(a,h)anthracene	53-70-3		1
		Dibenzothiophene	132-65-0		1
		2,6-Dimethylnaphthalene	581-42-0		1
		Fluoranthene	205-99-2		1
		Fluorene	86-73-7		1
		Indeno(1,2,3-c,d)pyrene	193-39-5		1
		1-Methylnaphthalene	90-12-0		1
		2-Methylnaphthalene	91-57-6		1
		1-Methylphenanthrene	832-69-9		1
Naphthalene	91-20-3		1		
Perylene	198-55-0		1		
Phenanthrene	85-01-8		1		
Pyrene	129-00-0		1		
2,3,5-Trimethylnaphthalene	2245-38-7		1		

### 8.5.1 Data Entry

Required data elements that laboratories must provide to the USEPA, are identified in the USEPA’s data template, available separately from the USEPA. If the laboratory applies its own QC codes, the data transmittal must define the codes.

## 8.6 Pertinent QA/QC Procedures

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

### 8.6.1 QC Samples

Once or twice during the performance period (preferably once at the beginning and once at the end), the External QC Coordinator will 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 NLA 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 External QC Coordinator will compare the results to the expected value and determine consistency between laboratories (e.g., determine if one laboratory is 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 unique laboratory practices that might account for differences between the laboratory and others. The contractor shall analyze the external QC samples using the same procedures as those for the field samples.

### 8.6.2 Summary of QA/QC 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, matrix spike duplicate, laboratory duplicate, and method blank samples. Also, each laboratory QC samples (i.e., preparation and instrument blanks, laboratory control sample (LCS), spike/duplicate, etc.) must be given a unique sample identification. **Table 8-4** provides a summary of the quality control requirements.

**Table 8-4 Sediment chemistry, grain size, and TOC: quality control activities for samples.**

Activity	Evaluation	Corrective Action
Demonstrate competency for analyzing sediment samples to meet the performance measures	Demonstration of competency with sediment samples in achieving the method detection limits, accuracy, and precision targets.	The USEPA will not approve any laboratory for the NLA sample processing if the laboratory cannot demonstrate competency. In other words, the USEPA will select another laboratory that can demonstrate competency for its NLA samples.
Check condition of sample when it arrives.	Sample issues such as cracked containers; missing labels; insufficient volume for testing.	Assign appropriate condition code identified in Table 8-1. Error! Reference source not found..

Activity	Evaluation	Corrective Action
Store sample appropriately. While stored at the laboratory, the sample must be kept at a temperature $\leq -20^{\circ}\text{C}$ except jars for grain size analyses are refrigerated at $4^{\circ}\text{C}$ .	Check the temperature of the refrigerator/freezer and refrigerator per the laboratory's SOPs.	Record temperature of sample upon arrival at the laboratory. If at any other time, samples are warmer than required, note temperature and duration of deviation in comment field.  Data analyst will consider temperature deviations in evaluating the data. He/she will flag the deviations and determine whether the data appear to be affected and/or the data should be excluded from the analyses.
Analyze sample within holding time	The test must be completed within the holding time of 1 year. If the original test fails, then the retest also must be conducted within the holding time.	Perform test, but note reason for performing test outside holding time. The USEPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.
Perform once at the start of each batch to evaluate the labeled compound recovery (LCR) in a Laboratory Control Sample (LCS). This tests the performance of the equipment.	Control limits for recovery cannot exceed $100\pm 20\%$ .	First, prepare and analyze one additional LCS. If the second blank meets the requirement, then no further action is required. If the second LCS fails, then determine and correct the problem before proceeding with any sample analyses.
Perform once at the start of each batch to evaluate the entire extraction and analysis process using a Method Blank	Control limits cannot exceed the laboratory reporting level (LRL).	First, prepare and analyze one additional blank. If the second blank meets the requirement, then no further action is required. If the second blank fails, then determine and correct the problem (e.g., contamination, instrument calibration) before proceeding with any sample analyses. Reestablish statistical control by analyzing three blank samples. Report values of all blanks analyzed.
Check calibration immediately before and immediately after the sample batch (abbreviated as QCCS for quality control check sample)	Results must be $\pm 10\%$ of each other or as specified in method criteria	If calibration fails before analysis, recalibrate and reanalyze QCCS until it passes. If check fails after all samples in the batch have been analyzed, verify the QCCS reading. If the QCCS reading fails a second time, then reanalyze all samples in the batch and report only the set of results associated with the

SEDIMENT CONTAMINANTS, GRAIN SIZE, AND TOC

Activity	Evaluation	Corrective Action
		acceptable QCCS reading. Also report all QCCS readings for the batch.
Compare results of one laboratory duplicate sample (for TOC) or matrix spike duplicate sample (for contaminants) for each batch (not required for grain size)	Results must be within the target precision goal in <b>Section 8.5</b> .	If both results are below LRL, then conclude that the test has passed. Otherwise, prepare and analyze a split from different sample in the batch. If the second result is within the target precision goal (see <b>Section 8.5</b> ) of the original sample, then report the data and findings for both QC samples. However, if the two results differ by more than the target precision goal, review precision of QCCS measurements for batch; check preparation of split sample; etc. and report evaluation and findings in the case narrative. Consult with the USEPA HQ NLA Laboratory Review Coordinator to determine if reanalysis of the entire batch (at the laboratory's expense) is necessary. If no reanalysis is necessary, report and quantify all samples in batch. If reanalysis is necessary, then report all QC samples and the 2 <sup>nd</sup> analysis of the batch. If the second set also is unacceptable, then assign a QC code to each sample in the batch.
Compare results of one matrix spike sample per batch to evaluate performance in matrix (not required for TOC and grain size)	Evaluate performance after the first 3 batches; and then every subsequent batch. Ideally, control limits for recovery will not exceed the target accuracy goal, but this may not be realistic for all parameters with this matrix.	If both the original and duplicate results are below LRL, then conclude that the test has passed for the batch. Otherwise, if any results are not within the target accuracy goal for the first 3 batches, within 2 working days, contact the USEPA HQ NLA Laboratory Review Coordinator to discuss method performance and potential improvements. After achieving acceptable results or the USEPA's permission to continue, perform the test for every subsequent batch. For each batch, report the results from the original analysis and its duplicate and their RPD for TOC; the matrix spike, matrix spike duplicate,

Activity	Evaluation	Corrective Action
		RPD and %recovery for contaminants.
Compare results of TOC Certified Reference Material once per each batch	Value must be within 10% of the certified value.	If value is outside the acceptable range, analyze a second CRM. If the second CRM also is measured outside the acceptable range, then determine and correct the problem (e.g., contamination, instrument calibration) before reanalyzing all samples in the batch.
Maintain the required MDL targets identified in <b>Section 8.5</b> and <b>Table 8-3</b>	Evaluate for each sample	If MDL could not be achieved, then provide dilution factor or QC code and explanation in the comment field.
Participate in External Quality Control	Evaluate QC samples provided by the External QC Coordinator	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.
Maintain completeness	Completeness objective is 95% for all parameters.	Contact the USEPA HQ NLA Laboratory Review Coordinator immediately if issues affect laboratory's ability to meet completeness objective.

\***Section 1.2** provides contact information for the USEPA HQ NLA Laboratory Review Coordinator. Laboratories under contract to the USEPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the Laboratory Review Coordinator.

## 9.0 ATRAZINE PESTICIDE SCREEN

This method describes the application of enzyme linked immunosorbent assay (ELISA) to the determination of atrazine and related atrazine occurrence and concentration in surface water samples. The Abraxis magnetic particle atrazine kit is used for this analysis.

You will filter the lake water sample, add the filtered water to a disposable test tube with an enzyme conjugate, and then add paramagnetic particles with atrazine-specific antibodies. After allowing for a 15-minute reaction between the sample and reagents, you apply a magnetic field to the test tube that retains the paramagnetic particles (with atrazine and labeled atrazine bound to the antibodies on the particles in proportion to their original concentration) and allow the unbound reagents to be decanted. After decanting, wash the particles with the washing solution. You will detect the presence of atrazine and related atrazines by adding the color solution. After an incubation period, the reaction is stopped and stabilized by the addition of a dilute acid (Stopping Solution). Because the labeled atrazine (conjugate) was in competition with any unlabeled atrazine in the sample for the antibody sites, the color developed is inversely proportional to the concentration of atrazine in the sample. The detection limit for this method is 0.03 µg/L and the reporting limit is 0.05 µg/L.

Cold atrazine pesticide screen samples will be shipped on ice from the field crews to the contract batching laboratory. The contract batching laboratory will store samples in the refrigerator and send the batched samples to the analysis laboratory in coolers on ice. Samples will arrive in the analysis laboratory chilled and they can be held in a refrigerator or cold room for several weeks. Atrazine pesticide screen analysis laboratories will need to process samples in accordance with the time frame outlined in contractual agreements.

The methods listed below follow the methods used by Minnesota Pollution Control Agency (MPCA) based on the ELISA kit instructions.

### 9.1 Responsibility and Personnel Qualifications

All laboratory personnel are trained in advance in the use of equipment and procedures used during the implementation of this SOP. All personnel are responsible for complying with all of the QA/QC requirements that pertain to this indicator.

### 9.2 Precautions

The stopping solution contains diluted sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). Avoid contact of the stopping solution with skin and mucous membranes. If this reagent comes in contact with the skin, wash with water. Consult state, local, and federal regulations for proper disposal of all reagents.

#### 9.2.1 Storage and Stability

Store all reagents at 2-8°C. Do not freeze reagents. Before use, allow the solutions to reach room temperature (20-25°C). Reagents may be used until the expiration date on the box. The test tubes and the washing solution require no special storage condition and may be stored separately from the reagents.

### 9.3 Equipment

Abraxis Atrazine Kit (each kit contains Atrazine Antibody Coupled Paramagnetic Particles, Atrazine Enzyme Conjugate, Atrazine Standards, Control, Diluent/ Zero Standard, Color Solution, Stopping Solution, Washing Solution, and test tubes)

Precision pipets capable of delivering 250 and 500 µL and a 1.0 mL repeating pipet

Vortex mixer

Magnetic separation system

Photometer capable of readings at 450 nm

### 9.4 Sample Receipt

Because USEPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps upon receiving a delivery.

1. Report receipt of samples to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** of the manual for contact information).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
  - a. Verify that the sample IDs in the shipment match those recorded on the sample tracking form
  - b. Record the information in **Table 9-1** for the NARS IM Team, 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. *W*: Sample is warm (>4°), record the temperature in the comment field, and perform the assay
  - c. If any sample is damaged or missing, contact the USEPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).
3. Store samples in the refrigerator until sample preparation begins.
4. Maintain the sample tracking forms with the samples.

**Table 9-1 Atrazine login: required data elements.**

FIELD	FORMAT	DESCRIPTION
LABORATORY ID	text	Name or abbreviation for laboratory
DATE RECEIVED	MMDDYY	Date sample was received by laboratory
SITE ID	text	NLA 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 (>4°)
		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	

## 9.5 Procedure

### 9.5.1 Test preparation

1. Filter all lake water samples with a 0.2 µm filter (e.g., Anotop or Arcodisc) to remove particles.
2. If the atrazine concentration of a sample exceeds 5 ppb, you will need to repeat the test with a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/ Zero Standard or Sample Diluent (e.g., make a ten-fold dilution by adding 100 µL of the sample to 900 µL of Diluent/ Zero Standard). Mix the dilution thoroughly before assaying. Perform the assay according to the Assay Procedure and calculate the final results by multiplying the value obtained by the dilution factor.
3. Bring reagents to room temperature and thoroughly mix the antibody coupled paramagnetic particles before use.

### 9.5.2 Procedural notes and precautions

- A consistent technique is important for optimal performance. For the greatest precision, treat each tube in an identical manner.
- Add reagents directly to the bottom of the tube while avoiding contact between the reagents already added to the tube and the pipet tip. This will help assure consistent quantities of reagent in the test mixture.
- Avoid cross contamination and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and the pipet tips.
- Avoid foam formation during vortexing.
- Mix the antibody coupled paramagnetic particles just prior to pipeting.

### 9.5.3 Assay procedure

1. Label test tubes for standards, controls, and samples (**Table 9-2**).
2. Add 200 or 250  $\mu\text{L}$  of the appropriate standard, control, or sample to the test tube.
3. Add 250  $\mu\text{L}$  of Atrazine Enzyme Conjugate to each tube.
4. Mix the Atrazine Antibody Coupled Paramagnetic Particles thoroughly and add 500  $\mu\text{L}$  to each tube.
5. Vortex for 1 to 2 seconds minimizing foaming.
6. Incubate for 15 minutes at room temperature.
7. Separate in the Magnetic Separation System for two minutes.
8. Decant and gently blot all tubes briefly in a consistent manner.
9. Add 1mL of washing solution to each tube and allow them to remain in the magnetic separation unit for two minutes.
10. Decant and gently blot all tubes briefly in a consistent manner.
11. Repeat steps 9 and 10 one additional time.
12. Remove the rack from the separator and add 500  $\mu\text{L}$  of Color Solution to each tube.
13. Vortex for 1 to 2 seconds minimizing foaming.
14. Incubate for 20 minutes at room temperature.
15. Add 500  $\mu\text{L}$  of Stopping Solution to each tube.
16. Add 1 mL Washing Solution to a clean test tube. Use as a blank in Step 17.
17. Within 15 minutes after the addition of the stopping solution, read the absorbance at 450 nm with a photometer.

### 9.5.4 Results

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the  $\%B/B_0$  for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/ Zero Standard.
3. Construct a standard curve by plotting the  $\%B/B_0$  for each standard on the vertical logit (Y) axis versus the corresponding atrazine concentration on the horizontal logarithmic (X) axis.
4.  $\%B/B_0$  for controls and samples will then yield levels in ppb of atrazine by interpolation of the standard curve.

Some instrument manufacturers make photometers that allow for automatic calculation of calibration curves. Refer to instrument operating manuals for detailed instructions.

**Table 9-2 Test tube labeling for atrazine assay.**

Tube Number	Contents of Tube
1,2	Diluent/ Zero Standard, 0 ppb
3,4	Standard 1, 0.1 ppb
5,6	Standard 2, 1.0 ppb
7,8	Standard 3 5.0 ppb
9	Control
10	Sample 1
11	Sample 2
12	Sample 3

### 9.5.5 Data Entry

Required data elements that laboratories must provide to the USEPA, are identified in the USEPA’s data template, available separately from the USEPA. If the laboratory applies its own QC codes, the data transmittal must define the codes.

## 9.6 Pertinent QA/QC Procedures

### 9.6.1 Internal QC

1. A control solution at approximately 3 ppb of atrazine is provided in the atrazine kit. Include a control in every run and treat it in the same manner as an unknown sample.
2. Prepare and incubate one duplicate sample for every 10 samples analyzed.
3. Table 9-3 provides a summary of the quality control requirements.

**Table 9-3 Atrazine: quality control requirements.**

Quality Control Activity	Description and Requirements	Corrective Action
<b>Kit – Shelf Life</b>	Is within its expiration date listed on kit box.	If kit has expired, then discard or set aside for training activities.
<b>Kit – Contents</b>	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.
<b>Calibration</b>	All of the following must be met: <ul style="list-style-type: none"> <li>○ Standard curve must have a correlation coefficient of <math>\geq 0.99</math>;</li> <li>○ Average absorbance value, <math>\bar{A}_0</math>, for S0 must be <math>&gt; 0.80</math>; and</li> <li>○ Standards S0-S3 must have decreasing average absorbance values. That is, if <math>\bar{A}_i</math> is the average of the absorbance values for <math>S_i</math>, then the absorbance average values must be: <math>\bar{A}_0 &gt; \bar{A}_1 &gt; \bar{A}_2 &gt; \bar{A}_3</math></li> </ul>	If any requirement fails: <ul style="list-style-type: none"> <li>• Results from the analytical run are not reported.</li> <li>• All samples in the analytical run are reanalyzed until calibration provides acceptable results. At its discretion, the lab may consult with EPA for guidance on persistent difficulties with calibration.</li> </ul>
<b>Kit Control</b>	The average concentration value of the duplicates (or triplicate) must be within the range of 3 ppb +/- 10%.	If the requirement fails: <ul style="list-style-type: none"> <li>• Results from the analytical run are not reported</li> <li>• The lab evaluates its processes, and if appropriate, modifies its processes to correct possible contamination or other problems.</li> </ul>
<b>Negative Control</b>	The values for the negative control replicates must meet the following requirements:	

	<ul style="list-style-type: none"> <li>○ All concentration values must be &lt; 0.05 µg/L (i.e., the reporting limit)</li> </ul>	<ul style="list-style-type: none"> <li>● The lab reanalyzes all samples in the analytical run until the controls meet the requirements.</li> </ul>
<b>Sample Evaluations</b>	Samples are run in duplicate: requires 1 in 10 duplication of samples; Each duplicate pair must have %CV≤10% between its absorbance values.	If the requirement fails when applying the: <i>All</i> samples in the kit, not just the sample that failed, must be run a second time. No samples are to be run more than twice.
<b>Results Within Calibration Range</b>	If the result is less than the upper calibration range (i.e., 5.0 µg/L for undiluted samples), then the requirement is met.	If a result registers as “HIGH”, then record the result with a data flag of “HI.” If the result registers as ‘HIGH,’ then the sample must be diluted and re-run. If the sample is evaluated using a duplicate pair, if one or both results register as ‘HIGH’, then the sample must be diluted and re-run. No samples are to be run more than twice. The lab reports both the original and diluted sample results.
<b>External Quality Control Sample</b>	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.

### 9.6.2 External QC

1. Analyze 10 provided spiked samples (blind sample) provided by the USEPA HQ Laboratory Review Coordinator. After processing the samples, the laboratory will send the results to the USEPA HQ Laboratory Review Coordinator. The results will be compared to the known concentrations and a determination made.

## 10.0 WATER CHEMISTRY and CHLOROPHYLL A

### 10.1 Analytical Parameters

A total of 19 parameters are determined from each bulk water chemistry sample collected (**Table 10-1**). In addition, chlorophyll-*a* is determined from a separate, discrete sample following the same performance-based methods approach as proposed for water chemistry analytes.

**Table 10-1 Water chemistry parameters measured for the National Lakes Assessment 2017.**

Analyte	Units	Comments
Conductivity	µS/cm at 25°C	
pH	Standard (Std) Units	
Turbidity	NTU	
Acid Neutralizing Capacity (ANC)	µeq/L (20 µeq/L=1 mg as CaCO <sub>3</sub> )	
Dissolved Organic Carbon (DOC)	mg /L	
Ammonia-N (NH <sub>3</sub> -N)	mg /L	The method measures ammonia and ammonium; the relative proportion between these two analytes depends on pH. Typically, NLA (and other NARS) samples consist of mostly ammonium
Nitrate-Nitrite (NO <sub>3</sub> -NO <sub>2</sub> )	mg/L	Note different preservation methods and holding times depending on whether the lab is using ion chromatography (IC) or flow injection analysis (FIA)
Total Nitrogen (TN)	mg/L	
Total Phosphorus (TP)	µg /L	
Sulfate (SO <sub>4</sub> )	mg /L	
Chloride (Cl)	mg /L	
Nitrate (NO <sub>3</sub> )	mg /L	May be obtained as part of nitrate-nitrite determination (use FIA to obtain nitrate-nitrite and nitrite separately, then calculate difference for nitrate), or as a direct measurement (e.g., ion chromatography)
Aluminum (Al)	mg /L	
Calcium (Ca)	mg /L	
Magnesium (Mg)	mg /L	
Sodium (Na)	mg /L	
Potassium (K)	mg /L	
Silica (SiO <sub>2</sub> )	mg /L	
True Color	PCU	Performance objectives based on use of visual estimation method
Chlorophyll- <i>a</i>	µg/L (in extract)	

## 10.2 Sample Receipt

Because USEPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps upon receiving a delivery.

1. Report receipt of samples to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** of the manual for contact information).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
  - a. Verify that the sample IDs in the shipment match those recorded on the sample tracking form
  - b. Record the information in **Table 10-2** for the NARS IM Team, 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. *W*: Sample is warm (>7°), record the temperature in the comment field, and perform the assay
  - c. If any sample is damaged or missing, contact the USEPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).
3. Store samples in the refrigerator until sample preparation begins.
4. Maintain the sample tracking forms with the samples.

**Table 10-2 Water Chemistry login: required data elements.**

FIELD	FORMAT	DESCRIPTION	
LABORATORY ID	text	Name or abbreviation for laboratory	
DATE RECEIVED	MMDDYY	Date sample was received by laboratory	
SITE ID	text	NLA 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 (>7°)
		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	

### 10.3 Sample Processing and Preservation

Due to the short holding time of these samples, samples will be shipped overnight by the field crews and must be preserved by close of business (COB) the day after sample collection. If expected samples do not arrive or arrive after the acceptable time frame (24 hours after the samples were collected), laboratories must flag those samples on the sample check-in spreadsheet provided by the NARS IM Team and notify the NARS IM Coordinator (see **Section 1.2**).

Upon receipt of samples, inspect each sample and review the tracking form that was included with the samples. Samples damaged during the shipping process are flagged by the laboratory on the sample check-in spreadsheet upon receipt and inspection. Store samples at 4°C in darkness until aliquots are ready to be prepared. If possible, prepare aliquots the same day as samples are received, but no later than 48 hours after receipt. Laboratories should be familiar with and ensure that samples meet all defined target holding times. Any sample that does not meet holding time requirements is flagged and evaluated to determine if the exceedance impacts either sample integrity or any potential end uses of the data (USEPA 2002). Results from samples that exceeded target holding times are not rejected outright.

### 10.3.1 Water Chemistry Samples

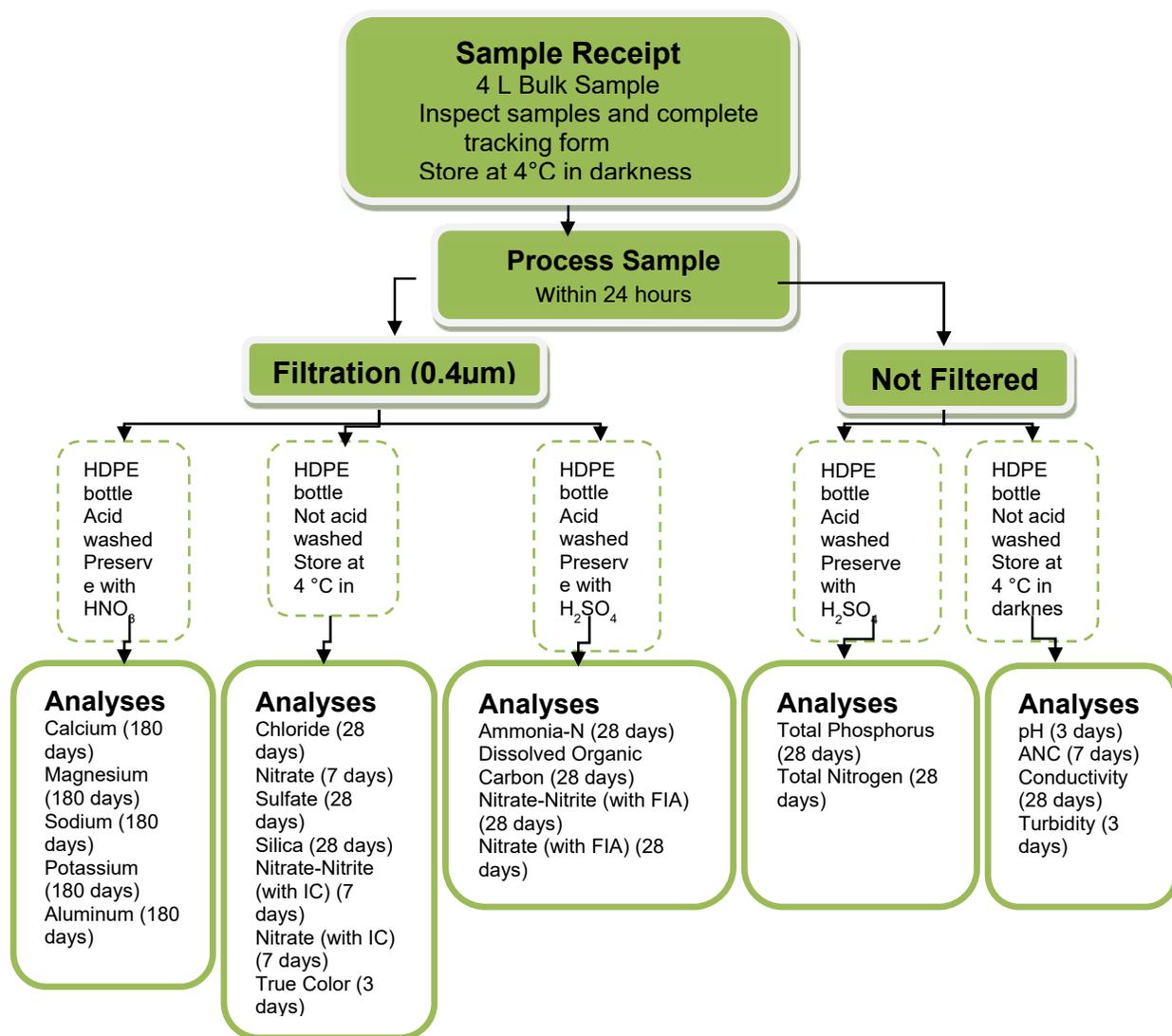


Figure 10.1 Water chemistry sample processing procedures.

Figure 10.1 illustrates sample preparation processing for the water chemistry indicators, including filtering and acidifying, for the various analytes.

1. Use 0.4µm pore size polycarbonate filters for all filtration.
2. Rinse vacuum filter funnel units thoroughly with reverse-osmosis (RO) or deionized (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. After all filtered and unfiltered aliquots are collected, add ultra-pure acid ( $\text{HNO}_3$  or  $\text{H}_2\text{SO}_4$ , depending on the analyte, see **Table 10-3**) to the sample in the aliquot container. Cap tightly and invert the bottle several times to mix.
5. Store all aliquots except the cation aliquot (filtered, acidified with  $\text{HNO}_3$ ) in a refrigerator at  $4^\circ\text{C}$ .

**Table 10-3 Acid preservatives added for various analytes.**

Preservatives	
$\text{H}_2\text{SO}_4$	$\text{HNO}_3$
DOC	Al
$\text{NH}_3\text{-N}$	Ca
Total N	Mg
Total P	Na
$\text{NO}_2\text{-NO}_3$	K

### 10.3.2 Chlorophyll-a Samples

Chlorophyll-*a* samples are filtered in the field, placed in a labeled centrifuge tube in a dark cooler, and stored on ice until arrival at the laboratory. Store the filter in the centrifuge tube in the freezer at  $-20 \pm 2^\circ\text{C}$  for no more than thirty days before analysis.

## 10.4 Performance-based Methods

As an alternative to specifying laboratory methods for sample analysis, a performance-based approach that defines a set of laboratory method performance requirements for data quality is utilized for this survey. Method performance requirements for this project identify lower reporting limit (LRL), precision, and bias objectives for each parameter (**Table 10-5**). The LRL is the lowest value that needs to be quantified (as opposed to just detected), and represents the value of the lowest non-zero calibration standard used. It is set to double the long-term method detection limit (LT-MDL), following guidance presented in Oblinger, Childress et al. (1999).

Precision and bias objectives are expressed in both absolute and relative terms following Hunt and Wilson (1986). The transition value is the value at which performance objectives for precision and bias switch from absolute ( $\leq$  transition value) to relative ( $>$  transition value). For pH, the objectives are established for samples with lower  $\text{H}^+$  (or  $\text{OH}^-$ ) concentrations (pH between 5.75 and 8.25) and higher  $\text{H}^+$  (or  $\text{OH}^-$ ) concentrations (pH  $<$  5.75 or  $>$  8.25).

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 (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. Bias (i.e., systematic error) 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.

Analytical methods used at the central laboratory (USEPA ORD-Corvallis) are summarized in **Table 10-4**. Participating laboratories may use alternative analytical methods for each target analyte if they can satisfactorily demonstrate the alternative method can achieve the performance requirements as listed in **Table 10-5**. Information is provided by the laboratory to the NLA Quality Team. The team reviews the information to determine whether the laboratories meet the necessary requirements. The information from this process is maintained in the NLA 2017 QA files by the USEPA HQ Laboratory Review Coordinator.

**Table 10-4 Summary of analytical methods used by NLA 2017 (Central Laboratory, USEPA ORD-Corvallis).**

Analyte	Summary of Method <sup>h</sup>	References <sup>i</sup>	WRS SOP <sup>j</sup>
pH (laboratory)	Automated, using ManSci PC-Titrate w/ Titra-Sip autotitrator and Ross combination pH electrode. Initial pH determination for ANC titration	USEPA 150.6 (modified)	WRS 16A.0 (April 2011)
Specific conductance @ 25°C	Electrolytic, Man-Tech TitraSip automated analysis OR manual analysis, electrolytic	USEPA 120.6	WRS 16A.0 (April 2011) WRS 11A.4 (April 2011)
Acid neutralizing capacity (ANC)	Automated acidimetric titration to pH≤3.5, with modified Gran plot analysis	U.S. USEPA (1987)	WRS 16A.0 (April 2011)
Turbidity	Nephelometric; Man-Tech TitraSip automated analysis, OR Manual analysis using Hach turbidimeter (high turbidity samples)	APHA 214 A, USEPA 180.1 U.S. EPA (1987)	WRS 16A.0 (April 2011) WRS 13A.3 (April 2011)
True color (Hach Kit)	Visual comparison to calibrated glass color disk.	APHA 204 A (modified), USEPA 110.2 (modified), U.S. EPA (1987)	WRS 15A.3 (April 2011)
Dissolved Organic Carbon (DOC) <sup>k</sup>	UV promoted persulfate oxidation to CO <sub>2</sub> with infrared detection	APHA 5310-C U.S. EPA (1987)	WRS 21A.4 (May 2011)
Nitrate+Nitrite, as N (fresh waters)	Ion Chromatography OR FIA automated colorimetric (cadmium reduction)	USEPA 300.6; SW-846 9056A; APHA 4110B  USEPA 353.2 APHA 4500-NO <sub>3</sub> -N-E Lachat 10-107-04-1-C	WRS 36A.0 (April 2011)  WRS 40A.5 (May 2011)
Nitrate	Measured as part of Nitrate-Nitrite when using IC or calculated after measuring Nitrate+Nitrite using FIA.	See above	See above
Ammonia, as N (fresh waters)	FIA automated colorimetric (salicylate, dichloroisocyanurate)	Lachat 10-107-06-3-D	WRS 30A.4 (April 2011)

<sup>h</sup> FIA=Flow injection analysis. AAS=Atomic Absorption Spectrometry

<sup>i</sup> 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.

<sup>j</sup> WRS= Willamette Research Station. References are to laboratory SOP being used at central laboratory. Available upon request. (contact the Project Lead)

<sup>k</sup> 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).

Silica, dissolved (SiO <sub>2</sub> ) Fresh waters	FIA automated colorimetric (molybdate, stannous chloride)	EPA366.0, APHA 425 C Lachat 10-114-27-1-B	WRS 32A.5 (February 2010)
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; Automated Colorimetric Analysis (molybdate, ascorbic acid)	APHA 4500-P-E USGS I-4650-03 U.S. EPA (1987) Lachat 115-01-1-B (modified)	WRS 34A.5 (April 2011)
Major anions, dissolved chloride, nitrate, nitrite, sulfate	Ion Chromatography	USEPA 300.6; SW-846 9056A; APHA 4110B	WRS 40A.5 (May 2011)
Major cations, dissolved calcium, sodium, potassium, magnesium, aluminum	Inductively-coupled Plasma Atomic Emission Spectroscopy (ICP-AES) OR Flame AAS	USEPA 200.7; USEPA 6010B  U.S. EPA (1987), USEPA 215.1 USEPA 273.1, USEPA 258.1 USEPA 242.1	WRS SOP 3.04 v3 (October 2011) WRS 50A.4 (March 2007)
Chlorophyll-a (Chl-a)	Extraction 90% acetone analysis by fluorometry	USEPA 445.0, USEPA 446.0	WRS 71A.3 (April 2011)

## 10.5 Pertinent QA/QC Procedures

A single central laboratory and some State laboratories will analyze the water chemistry samples. The specific quality control procedures used by each laboratory are implemented to ensure that:

- Objectives established for various data quality indicators being met.
- Results are consistent and comparable among all participating laboratories.

The central laboratory demonstrated in previous studies that it can meet the required LRL (USEPA 2004). QA/QC procedures outlined in this manual and the NLA 2017 QAPP will be followed to ensure these LRLs are met for the NLA 2017.

### 10.5.1 Laboratory Performance Requirements

**Table 10-5** summarizes the pertinent laboratory performance requirements for the water chemistry and chlorophyll-a indicators.

### 10.5.2 Laboratory Quality Control Samples

**Table 10-6** summarizes the pertinent laboratory quality control samples for the water chemistry and chlorophyll-a indicators.

**Table 10-5 Laboratory method performance requirements for water chemistry and chlorophyll-*a* sample analysis.**

Analyte	Units	Potential Range of Samples <sup>l</sup>	Lower Reporting Limit <sup>m</sup>	Transition Value <sup>n</sup>	Precision Objective <sup>o</sup>	Bias Objective <sup>p</sup>
Conductivity	µS/cm at 25°C	1 to 15,000	2.0	20	± 2 or ±10%	± 2 or 5%
pH (laboratory)	Std Units	3.5 to 10	N/A	5.75, 8.25	±0.07 or ±0.15 >5.75 and < 8.25: ±0.15	±0.05 or ±0.10 >5.75 and < 8.25: ±0.15
Turbidity	NTU	0 to 44,000	2.0	20	± 2 or ±10%	± 2 or ±10%
Dissolved Organic Carbon (DOC)	mg /L	0.1 to 109	0.20	≤ 1 > 1	± 0.10 or ±10%	± 0.10 or ±10%
Ammonia as N(NH <sub>3</sub> -N)	mg /L	0 to 17	0.02 (1.4 µeq/L)	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Nitrate-Nitrate (NO <sub>3</sub> -NO <sub>2</sub> )	mg /L	0 to 360 (as nitrate)	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Nitrogen (TN)	mg/L	0.1 to 90	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Phosphorus (TP)	µg /L	0 to 22,000	4	20	± 2 or ±10%	± 2 or ±10%

<sup>l</sup> Estimated from samples analyzed at the WED-Corvallis laboratory between 1999 and 2005 for TIME, EMAP-West, and WSA streams from across the U.S.

<sup>m</sup> The lower reporting limit is the lowest value that needs to be quantified (as opposed to just detected), and represents the value of the lowest nonzero calibration standard used. It is set to 2 times the long-term detection limit, following USGS Open File Report 99-193 *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*.

<sup>n</sup> Value at which performance objectives for precision and bias switch from absolute (≤ transition value) to relative (> transition value). Two-tiered approach based on Hunt, D.T.E. and A.L. Wilson. 1986. *The Chemical Analysis of Water: General Principles and Techniques*. 2<sup>nd</sup> ed. Royal Society of Chemistry, London, England.

<sup>o</sup> 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.

<sup>p</sup> Bias (systematic error) 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.

Sulfate (SO <sub>4</sub> )	mg /L	0 to 5,000	0.50 (10 µeq/L)	2.5	± 0.25 or ±10%	± 0.25 or ±10%
Chloride (Cl)	mg /L	0 to 5,000	0.20 (6 µeq/L)	1	± 0.10 or ±10%	± 0.10 or ±10%
Nitrate (NO <sub>3</sub> )	mg /L	0 to 360	0.02 (4 µeq/L)	0.1	± 0.01 or ±10%	± 0.01 ±10%
Calcium (Ca)	mg /L	0.04 to 5,000	0.10 (5 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Magnesium (Mg)	mg /L	0.1 to 350	0.10 (8 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Sodium (Na)	mg /L	0.08 to 3,500	0.10 (4 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Potassium (K)	mg /L	0.01 to 120	0.10 (2 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Silica (SiO <sub>2</sub> )	mg /L	0.01 to 100	0.10	0.5	± 0.05 or ±10%	± 0.05 or ±10%
True Color	PCU	0 to 350	5	50	±5 or ±10%	±5 or ±10%
Chlorophyll a	µg/L (in extract)	0.7 to 11,000	0.5	15	± 1.5 or ±10%	± 1.5 or ±10%

**Table 10-6 Laboratory quality control samples: water chemistry indicator.**

QC Sample Type and Description	Analytes	Description	Frequency	Acceptance Criteria	Corrective Action
<b>Laboratory/ Reagent Blank</b>	All		Once per day prior to sample analysis	Control limits $\leq$ LRL	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.
<b>Filtration Blank</b>	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.
<b>LT-MDL Limit Quality Control Check Sample (QCCS)</b>	All analyses except true color and turbidity	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.
<b>Calibration QCCS</b>	For turbidity, a QCCS is prepared at one level for routine analyses (USEPA 1987). Additional QCCSs are prepared as needed for samples having estimated turbidities greater than 20 NTU.		Before and after sample analyses	$\pm 10\%$ or method criteria	Repeat QCCS analysis. Recalibrate and analyze QCCS. Reanalyze all routine samples (including performance efficiency and field replicate samples) analyzed since the last acceptable QCCS measurement.

<b>Laboratory Duplicate Sample</b>	All analyses		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.
<b>Standard Reference Material (SRM)</b>	When available for a particular analyte		One analysis in a minimum of five separate batches	Manufacturers certified range	Analyze standard in next batch to confirm suspected imprecision or bias. 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.
<b>Matrix Spike Samples</b>	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).

### 10.5.3 Data Reporting, Review, and Management

Checks made of the data in the process of review and verification are summarized in **Table 10-7**. Data reporting units and significant figures are given in **Table 10-8**. The NLA 2017 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 10-7 Data validation quality control for water chemistry indicator.**

Activity or Procedure	Requirements and Corrective Action
<b>Range checks, summary statistics, and/or exploratory data analysis</b> (e.g., box and whisker plots)	Correct reporting errors or qualify as suspect or invalid.
<b>Review holding times</b>	Qualify value for additional review
<b>Ion balance:</b> Calculate percent ion balance difference (%IBD) using data from cations, anions, pH, and ANC. See <b>Equation 10.1</b> .	<ul style="list-style-type: none"> <li>• If total ionic strength <math>\leq 100 \mu\text{eq/L}</math> – %IBD <math>\leq \pm 25\%</math>.</li> <li>• If total ionic strength <math>&gt; 100 \mu\text{eq/L}</math> – %IBD <math>\leq \pm 10\%</math>.</li> <li>• Determine which analytes, if any, are the largest contributors to the ion imbalance. Review suspect analytes for analytical error and reanalyze. – Flag = unacceptable %IBD</li> <li>• If analytical error is not indicated, qualify sample to attribute imbalance to unmeasured ions. Reanalysis is not required. – Flag = %IBD outside acceptance criteria due to unmeasured ions</li> </ul>
<b>Conductivity check:</b> Compare measured conductivity of each sample to a calculated conductivity based on the equivalent conductance of major ions in solution (Hillman et al., 1987)	<ul style="list-style-type: none"> <li>• If measured conductivity <math>\leq 25 \mu\text{S/cm}</math>, – <math>([\text{measured} - \text{calculated}] \div \text{measured}) \leq \pm 25\%</math>.</li> <li>• If measured conductivity <math>&gt; 25 \mu\text{S/cm}</math>, – <math>([\text{measured} - \text{calculated}] \div \text{measured}) \leq \pm 15\%</math>.</li> <li>• Determine which analytes, if any, are the largest contributors to the difference between calculated and measured conductivity.</li> <li>• Review suspect analytes for analytical error and reanalyze.</li> <li>• If analytical error is not indicated, qualify sample to attribute conductivity difference to unmeasured ions. Reanalysis is not required.</li> </ul>
<b>Review data</b> from QA samples (laboratory PE samples, and inter-laboratory comparison samples)	Indicator QC Coordinator determines impact and possible limitations on overall usability of data based on the specific issue.

**Table 10-8 Data reporting criteria: water chemistry indicator.**

Measurement	Units	No. Significant Figures	Maximum No. Decimal Places
<b>DO</b>	mg/L	2	1
<b>Temperature</b>	°C	2	1
<b>pH</b>	pH units	3	2
<b>Carbon, dissolved organic</b>	mg/L	3	1

<b>ANC</b>	µeq/L	3	1
<b>Conductivity</b>	µS/cm at 25 °C	3	1
<b>Aluminum, calcium, magnesium, sodium, potassium, chloride, nitrate, and sulfate</b>	µeq/L	3	1
<b>Silica</b>	mg/L	3	2
<b>Total phosphorus</b>	µg/L	3	0
<b>Total nitrogen</b>	mg/L	3	2
<b>Nitrate-Nitrite</b>	mg/L	3	2
<b>Ammonia-N</b>	mg/L	3	2
<b>Turbidity</b>	NTU	3	0
<b>True color</b>	PCU	2	0
<b>Chlorophyll a</b>	ug/l	3	2

The ion balance for each sample is computed using the results for major cations, anions, and the measured acid neutralizing capacity. The percent ion difference (%IBD) for a sample is calculated as:

**Equation 10.1 Percent ion difference (%IBD)**

$$\%IBD = \frac{(\sum cations - \sum anions) - ANC}{ANC + \sum anions + \sum cations + 2[H^+]}$$

where ANC is the acid neutralization capacity; cations are the concentrations of calcium, magnesium, sodium, potassium, and ammonium (converted from mg/L to µeq/L); anions are the concentrations of chloride, nitrate, and sulfate (converted from mg/L to µeq/L), and H<sup>+</sup> is the hydrogen ion concentration calculated from the antilog of the sample pH. Factors to convert major ions from mg/L to µeq/L are presented in **Table 10-9**.

For the conductivity check, equivalent conductivities for major ions are presented in **Table 10-10**.

**Table 10-9 Constants for converting major ion concentration from mg/L to µeq/L**

Analyte	Conversion from mg/L to µeq/L <sup>q</sup>
Calcium	49.9
Magnesium	82.3
Potassium	25.6
Sodium	43.5
Ammonia-N	55.4
Chloride	28.2
Nitrate	16.1
Sulfate	20.8

<sup>q</sup> Measured values are multiplied by the conversion factor.

**Table 10-10 Factors to calculate equivalent conductivities of major ions.<sup>r</sup>**

Ion	Equivalent Conductance per mg/L (μS/cm at 25 °C)	Ion	Equivalent Conductance per mg/L (μS/cm at 25 °C)
Calcium	2.60	Nitrate	1.15
Magnesium	3.82	Sulfate	1.54
Potassium	1.84	Hydrogen	3.5 x 10 <sup>5s</sup>
Sodium	2.13	Hydroxide	1.92 x 10 <sup>5</sup>
Ammonia-N	4.13	Bicarbonate	0.715
Chloride	2.14	Carbonate	2.82

#### 10.5.4 Data Entry

Required data elements that laboratories must provide to the USEPA, are identified in the USEPA's data template, available separately from the USEPA. If the laboratory applies its own QC codes, the data transmittal must define the codes.

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<sup>r</sup> From Hillman et al. (1987).

<sup>s</sup> Specific conductance per mole/L, rather than per mg/L.

## 11.0 ZOOPLANKTON METHODS

This method is used to identify and enumerate species of lake zooplankton collected with vertical plankton net tows using the NLA 2017 method. Macrozooplankton are counted from a sample using a 150 µm mesh nets. Microzooplankton, especially rotifers, nauplii, copepodites <0.6 mm long, and cladocerans <0.2 mm long, are counted from a sample collected using a 50 µm mesh nets.

Zooplankton samples will be preserved in the field with EtOH and shipped from field crews to a contract batching laboratory. The contract batching laboratory will send the batched samples to the analysis laboratory. Preserved samples can be held for several months, but zooplankton analysis laboratories will need to process samples in accordance with the time frame outlined in contractual agreements. Contractual agreements for delivery of data do not supersede indicator holding times.

### 11.1 Responsibility and Personnel Qualifications

This procedure may be used by any person who has received training in processing and/or identification of zooplankton samples. It is also important that the taxonomist maintains contact with other taxonomists through professional societies and other interactions, and keep abreast of the pertinent literature, because taxonomic groupings and nomenclatural basis for taxonomy and nomenclature are updated frequently. A second taxonomist will re-identify a randomly-selected 10% of the samples for QC, as noted below, to quantify taxonomic precision, or consistency, as **percent taxonomic disagreement (PTD)**, help target corrective actions, and ultimately help minimize problems during data analysis. Samples are sent from the field to the laboratory on a regular basis during the project to avoid delays in processing and specimen identification.

### 11.2 Precautions

Wear appropriate clothing for safety precautions, such as nitrile gloves, rubber apron, long pants, etc. Follow all laboratory safety and waste disposal guidelines regarding the disposal of formalin (37% formaldehyde) solutions.

### 11.3 Equipment/Materials

- Dissection microscope (magnifications: 10X-50X)
- Compound microscope (magnifications: 40X-400X with phase-contrast capability)
- Hensen-Stempel pipettes (1, 2, and 5 mL)
- Graduated cylinders (100-, 250-, and 500mL)
- Folsom plankton Splitter
- Ward counting wheel or other suitable counting chamber
- Utermöhl counting chamber or Sedgwick-Rafter counting cell (1 mL vol) with cover slips
- Ring nets with 50, 500 and 1000 µm Nitex mesh
- Mechanical or electronic tally counters
- Microscope slides, 1 x 3 inch
- Cover slips
- Tubes for concentrating plankton samples (see below)
- Small sieves with 45 and 140-µm mesh
- 50-µm Nitex mesh Heavy duty rubber bulb Microprobe

150- $\mu$ m Nitex mesh Heavy duty rubber bulb Microprobe  
Micro-forceps  
100- to 500-mL glass jars with split fractions written on labels  
Zooplankton Sample Log-In Form  
Zooplankton Laboratory Sheet  
Labels

Construct the first plankton concentrating tube by covering one end of a wide glass tube (such as a chromatography tube) with 50- $\mu$ m mesh. Secure the mesh with O-rings and attach a heavy-duty bulb to the other end to provide suction. Construct the second plankton concentrating tube by covering one end of a wide glass tube (such as a chromatography tube) with 150- $\mu$ m mesh. Secure the mesh with O-rings and attach a heavy-duty bulb to the other end to provide suction.

The following reagents are needed:

- Formalin (37% formaldehyde solution)
- 95% EtOH
- 5% Sodium hypochlorite solution (unscented bleach)
- Rose Bengal stain dissolved in EtOH
- Dilute solution of laboratory detergent

#### 11.4 Sample Receipt

Because USEPA 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 to the NARS IM Team by completing and emailing the sample tracking spreadsheet with the sample login and sample condition information. (See **Section 1.2** of the manual for contact information).
2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
  - a. Verify that the sample IDs in the shipment match those recorded on the sample tracking form.
  - b. Record the information in **Table 11-1** for the NARS IM Team, 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
  - c. If any sample is damaged or missing, contact the USEPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).
3. Store samples until sample preparation begins.
4. Maintain the sample tracking forms with the samples.

**Table 11-1 Zooplankton login: required data elements.**

FIELD	FORMAT	DESCRIPTION	
LAB	text	Name or abbreviation for laboratory	
DATE RECEIVED	MMDDYY	Date sample was received by laboratory	
SITE ID	text	NLA 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
		Q	Other quality concerns, not identified above
CONDITION COMMENT	text	Comments about the condition of the sample.	

## 11.5 Procedure

### 11.5.1 Zooplankton Stratified Splitting

- Record all zooplankton samples received at the laboratory in a log book or sample log form (See **APPENDIX B: SAMPLE LABORATORY FORMS: Zooplankton Sample Log In Form**). Add approximately 1 to 3 mL of Rose Bengal stain solution to each sample bottle to aid in finding the smaller organisms. Process samples one at a time. Shake jar to mix water sample. Under the hood, rinse the first sample jar taken with the 50- $\mu$ m mesh net through a 45- $\mu$ m mesh sieve with deionized (DI) water to remove the EtOH; the second sample bottle, taken from the 150- $\mu$ m mesh net, is rinsed through a 145- $\mu$ m mesh sieve with DI water to remove the EtOH. The two mesh size samples are treated as individual samples for processing and identification and are to be recorded on the laboratory bench sheet with the sample number and corresponding mesh size.
- Be sure to rinse the corresponding sample bottles thoroughly with reverse osmosis (RO)/DI/distilled water into the 45- $\mu$ m mesh and 145- $\mu$ m mesh sieve to remove any residual organisms adhering to walls of the bottle. Rinse all containers from which zooplankton are transferred thoroughly, including the Folsom splitter, glass jars, and counting chambers. Wash the sample into a glass jar. Add a small amount of dilute laboratory soap to each sample, at this time, to prevent organisms from sticking to the sides of the containers and from floating at the

surface of the sample.

3. Stir the sample gently to break up algal clumps and then pour the entire sample into the Folsom plankton splitter. Stir the sample again to distribute animals uniformly and split the sample by immediately rotating the splitter before the organisms can settle. Rinse the inside of the splitter well to remove organisms that may stick to the sides. Rinse one sub-sample from the splitter receiving trays and save it in a labeled jar indicating the fraction of total original volume of sample bottle (1/2).
4. Place the second sub-sample from the split in the Folsom plankton splitter and divide again. Save one sub-sample in a labeled jar indicating the fraction of the total original volume it contains (1/4).
5. Repeat Steps 3 and 4 as many times as necessary until the last 2 sub-samples contain at least total of 400, and a maximum of 480 (400+20%), macrozooplankton each (not including rotifers and nauplii). These 2 sub-samples represent equal fractions of the original sample. Save one sub-sample in a jar labeled "A", and save the other sub-sample in a jar labeled "B". This process may vary depending on the density of organisms in the sample. If the minimum count is reached in the "A" subsample, then there is no need to identify individuals from subsample "B". Write the final split factor used, on the identification and enumeration bench sheets (see **APPENDIX B: SAMPLE LABORATORY FORMS: Zooplankton Enumeration Data Sheet** in).

## 11.5.2 Taxonomy Procedures

### 11.5.2.1 Taxonomic Level of Effort

The USEPA will supply a list of taxa that have been collected from previous iterations of the National Lakes Assessment (provided during laboratory initiation call). This list should be used as a guide for the appropriate taxa names to be used while processing samples. However, this list will clearly not include all potential taxa that may be encountered, but should assist in ensuring consistency between surveys. When possible the following resources should be used to identify zooplankton to species: Edmondson (1959), Pennak (1978), Smith and Fernando (1978), Stemberger (1979), the online Free-living and Parasitic Copepods (Including Branchiurans) of the Laurentian Great Lakes: Keys and Details on Individual Species and the online Image-Based Key to the Zooplankton of the Northeast, USA, produced by the University of New Hampshire Center for Freshwater Biology ([cfb.unh.edu](http://cfb.unh.edu)). Other resources and keys can be utilized, but should be provided to USEPA before processing of samples begin.

### 11.5.2.2 Macrozooplankton Identification and Enumeration (Excluding Rotifers and Nauplii)

Macrozooplankton are counted and identified from samples collected with the coarse mesh (150 µm) plankton net.

- 1.0 Species-level resolution will be the taxonomic requirement for macrozooplankton.
- 2.0 The taxonomist must examine and enumerate as many sub-samples needed to reach the target count of 400 to 480 organisms and record the information on the appropriate form (see **APPENDIX B: SAMPLE LABORATORY FORMS: Zooplankton Enumeration Data Sheet**).
- 3.0 Concentrate the sub-sample by using the small sieve or the condensing tube and place in a circular (or other suitable) counting chamber.
- 4.0 Identify all macrozooplankton under a dissecting microscope and enumerate using a mechanical or electronic tally counter.
- 5.0 Count the first two sub-samples which likely contain 400 organisms (**Section 11.5.1**, step 5) first, and count additional subsamples to reach enumeration target, if need. Examine and enumerate all macrozooplankton. If the minimum of 400 organisms in the first of

the two original subsamples, then stop. There will be no need to examine the second of the first two subsamples. During identification and enumeration, make measurements on selected individuals. For dominant taxa, measure a minimum of 20 individuals. For subdominant taxa (taxa encountered less than 40 times during enumeration), measure 10 individuals. For rare taxa (taxa encountered less than 20 times during enumeration), measure 5 individuals. If rare taxa are in a position that makes it difficult to measure (e.g. odd angle), then remove these individuals after identification and enumeration and measure them separately. Additionally, while enumerating and identifying samples, especially note invasive species such as *Bythotrephes* and *Cercopagis*.

#### 11.5.2.2.1 General Analysis and Guidelines

1. Mount organisms requiring higher magnification for identification on slides and examine at 100 - 1000x magnification under a compound microscope.
2. While counting macrozooplankton, make sure that all organisms are settled to the bottom. It is possible to sink floating macrozooplankton by gently pressing them down using the microprobe or by adding a drop of dilute laboratory detergent.
3. If a sample cannot be completely counted and archived within 2 days, keep the sample in the refrigerator and add a few drops of formalin to the jars to prevent organisms from clumping. Sample analysis should not extend beyond four days.
4. Place voucher specimens in a labeled vial and preserve with 95% EtOH. The label in the vial should include genus/species name, date preserved, analyst initials, station number, and sample number. A second taxonomist should confirm the voucher specimens.

#### 11.5.2.2.2 Large Taxa Scan

Observe non-counted sample portion for the following: *Leptodora*, *Chaoborus*, *Craspedacusta sowerbii*, *Mysidae*, *Ostracoda*, and *Hydracarina*. Spend minimal effort here, < ~1-2 minutes. If detected, enter "yes" in the LARGE\_RARE column on spreadsheet, and put the number counted in the L/R\_AUND column.

#### 11.5.2.3 Microzooplankton (Rotifers, Nauplii, and Crustaceans)

Microzooplankton are counted and identified from samples collected with the fine mesh (50 µm) plankton net.

1. Species-level resolution is the taxonomic requirement for rotifers, copepods <0.6 mm long, and *cladocerans* <0.2 mm long. Nauplii will be identified to the lowest possible taxonomic unit.
2. Selection of the split level from which a sub-sample for rotifer enumeration is based on estimates made during macrozooplankton enumeration (rotifers and small crustaceans are visible in the dissecting microscope).
3. Take two separate 1-mL sub-samples from the appropriate split. Count and identify microzooplankton from these two sub-samples (see **Section 11.5.2.4**). In cases where abundances are particularly low, use more than one 1-mL sub-sample for each count (see step 6).
4. Mix the sample thoroughly, and withdraw a 1-mL sub-sample with a Hensen-Stempel pipette (or other pre-calibrated large-bore pipette).
5. The 1-mL sub-sample should contain 400 rotifers, crustacean, and nauplii.
6. If the sub-sample contains less than 400 organisms, take a different sub-sample from a jar with a larger fraction of the original sample volume. If the sub-sample contains more than 480 organisms, use another sub-sample from a jar with a smaller fraction.
7. It is also permissible to use a second 1-mL aliquot if the original aliquot has less than 400 organisms. Count this second aliquot in the same manner as the first and combine the results to

make a final count.

8. In cases of extremely low microzooplankton densities, concentrate the sample prior to taking sub-samples with the pipette. The maximum number of 1-mL aliquots counted at the lowest possible split level is 3 per count (i.e., a total of 6 mL), even if the sum does not reach 400 organisms.

#### 11.5.2.3.1 Preparation and Microzooplankton Enumeration

1. Place the sub-sample in an Utermöhl counting chamber or Sedgwick-Rafter cell and cover with a glass cover slip.
2. Identify and enumerate all rotifers, microzooplankton, nauplii, and *Dreissena* veligers and post-veligers under a compound microscope at 100x magnification. Record results on the appropriate form. Make measurements on selected individuals at this time, and follow dominate, subdominant, and rare (**Section 11.5.2.2**). See measurement parameters for macro- and microzooplankton in **Sections 11.5.2.4.1** and **11.5.2.4.2** respectively.
3. After the counts are completed, measure the volume of the split used, including the volume of the aliquots, and record this information.

#### 11.5.2.4 Measurement of Macrozooplankton and Microzooplankton

##### 11.5.2.4.1 Crustaceans

To determine size distribution, measure zooplankton by use of a calibrated eyepiece micrometer during the identification and enumeration process.

Measure the first 20 encounters per species per sample as follows:

*Cladocera*: Length from the top of the head to the base of the caudal spine or to the end of the carapace.

*Copepoda*: Length from tip of the head to the insertion of spines into the caudal ramus.

*Mysis*: Carapace length, or the length from the tip of the head to the cleft in the telson.

*Bythotrephes*: Body length, excluding the caudal process.

*Cercopagis*: Body length, from the top of the eye to the end of the caudal claws.

**NOTE:** *If the organisms are curved or bent, make several straight line measurements and sum to obtain total length.*

##### 11.5.2.4.2 Rotifers

Measure at least 20 encounters per species as follows:

1. Loricated forms: body length from corona to the opposite end at the base of spine (if present).
2. Non-loricated forms: body length from corona to the opposite end, excluding spines, paddles, "toes" or other extensions.

## 11.6 Calculating and Reporting

Report zooplankton densities as number of organisms per cubic meter, which is calculated in the following equations.

### 11.6.1 Volume of water filtered

Equation 11.1 Volume of water filtered.

$$V = L \times A$$

where:

- V = Volume of water filtered (m<sup>3</sup>)
- L = Length of vertical tow
- A = Area of the mouth of the net (m<sup>2</sup>) = 0.1963 m<sup>2</sup> for 0.5-m diameter net

### 11.6.2 Macrozooplankton Densities

**Equation 11.2 Microcrustacean densities.**

$$D = \frac{N \times S}{V}$$

where:

- D = Density of organisms in number per cubic meter
- N = Number of organisms
- S = Spilt factor
- V = Volume of water filtered (from above calculation)

### 11.6.3 Microzooplankton Densities

**Equation 11.3 Microzooplankton densities.**

$$D = \frac{(N \times V_s \times S)}{N_a \times V}$$

where:

- D = Density of organisms in number per cubic meter
- N = Number of organisms
- N<sub>a</sub> = Number of 1mL aliquots examined
- V<sub>s</sub> = Volume of sub-samples from which aliquots were taken
- S = Spilt factor
- V = Volume of water filtered (from above calculation)

### 11.6.4 Zooplankton Biomass Estimates

Biomass estimates will be based on established length/width relationships (Dumont et al. 1975; McCauley, 1984; Lawrence et al. 1987). The lengths or the lengths and widths of each species encountered will be measured and will be equal to 20 for common species and lesser for more rare taxa. For cladocerans, the length will be measured from the tip of the head to the end of the body (shell spines excluded). For copepods, the length will be determined from the tip of the head to the insertion of the caudal ramus. The length of rotifers will be measured from the tip of the head to the end of the body (spines, toes, etc. excluded). In accordance with McCauley (1984), biomass will be computed for the appropriate number of individuals for each sample location and the arithmetic mean biomass will be multiplied times the species abundance to produce a species biomass for each sample. More detailed discussion of the methodology is given in Havens et al (2011), Beaver et al. (2010), and Havens & Beaver (2010).

### 11.6.5 Results of Laboratory Processing, Sample Archiving

Prepare a completed data sheet with list of taxa and number of individuals of each taxon for each sample. In addition, you should organize and archive the full complement of specimens (in containers of preservative and/or on permanent slide mounts), the “counted” sample (in jars, vials, or slide mounts), the concentrated split sample, and the unused sample split/fraction. All sample components should be clearly-labeled to associate multiple vials and slides as a single sample. Labels should be as Sample ID

“A,” jar/vial 1 of x, and Sample ID “A,” slide 1 of x; and Sample ID “A,” unused sample fraction (1/2 original volume).

Required data elements that laboratories must provide to the USEPA, are identified in the USEPA’s data template, available separately from the USEPA. If the laboratory applies its own QC codes, the data transmittal must define the codes.

## 11.7 Pertinent QA/QC Procedures

**Table 11-2** provides a summary of quality assurance/quality control procedures for the zooplankton indicator.

### 11.7.1 Sorting and Subsampling QC

For each laboratory, approximately 10% of the samples are randomly-selected for evaluation of subsampling precision (consistency of duplicate processing) by the laboratory. For these samples, the unused fractions will be treated in an identical manner as the primary fractions (taxonomic identification and enumeration). There are two precision calculations, one for tracking error for individual samples, and the other for estimating error for the overall dataset. Differences between the two sample fractions are an indication of subsampling consistency, quantified by relative percent difference (RPD) as follows:

**Equation 11.4 Relative percent difference (RPD).**

$$RPD = \frac{|n_1 - n_2|}{(n_1 + n_2)/2} \times 100$$

where  $n_1$  is the metric or index value from the first subsample, and  $n_2$  is the metric or index value from the second. The magnitude of error expected to be associated with splitting zooplankton samples is unknown; thus a specific measurement quality objective is not proposed here. For estimating subsampling error for the overall dataset, root mean square error (RMSE) is calculated. Also called standard error of estimate, this statistic is an estimate of the standard deviation of a population of observations and is calculated by:

**Equation 11.5 Root mean square error (RMSE) or standard error of estimate.**

$$RMSE = \sqrt{\frac{\sum_{j=1}^k \sum_{i=1}^{n_j} (y_{ij} - \bar{y}_j)^2}{\sum df_{1...k}}}$$

where  $y_{ij}$  is the  $i^{\text{th}}$  individual observation in group  $j$ ,  $j = 1...k$  (Zar 1999). More simply put, the equation can be described as the root of the sums of squared residuals across all subsample pairs, divided by the number of sample pairs. For computational convenience, RMSE is often calculated by taking the root of the mean square error (MSE), which can be output from an analysis of variance (ANOVA).

### 11.7.2 Taxonomic QC

#### 11.7.2.1 Internal Taxonomic QC

As directed by the Indicator QC Coordinator, an in-house QC Analyst will randomly select 5 of the samples counted and identified by individual taxonomists to ensure that each meets the acceptable criteria for percent identification efficiency which is 90%.

If the individual fails to maintain a  $\geq 90\%$  identification as determined by QC checks, previous samples will be re-counted and identified. EPA may also calculate or work with the lab to calculate the proportional analysis found in the External Taxonomic QC section below using internal QC information.

### 11.7.2.2 External Taxonomic QC

1. EPA may implement an external taxonomic QC review process for zooplankton. If EPA implements an external QC process, upon receipt of the data after initial identification, approximately 10% of the samples (for each laboratory) are randomly-selected for evaluation of taxonomic precision by the Indicator QC Coordinator. Following primary identification and enumeration, the jars, vials, and slides for each of these samples are sent by the original laboratory to a QC taxonomist for complete re-identification and re-enumeration. The laboratory will complete and send with the samples a sample tracking form. Differences between the two samples are an indication of taxonomic precision.
2. Precision of taxonomic identifications is determined by calculating percent taxonomic disagreement (PTD) of taxonomic results from two independent taxonomists, using the formula:

**Equation 11.6 Percent taxonomic disagreement (PTD).**

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

where  $comp_{pos}$  is the number of agreements, and N is the total number of organisms in the larger of the two counts (Stribling et al. 2003).

3. A PTD of 15% or less is recommended for taxonomic difference (overall mean  $\leq 15\%$  is acceptable). Individual samples exceeding 15% are examined for taxonomic areas of substantial disagreement, and the reasons for disagreement investigated. A reconciliation call between the primary and secondary taxonomist will facilitate this discussion. Results greater than this value are investigated and logged for indication of error patterns or trends.
4. The calculation of PTD is dependent on the number of organisms reported by the taxonomist. Where there is potential for a large loss of organisms between the two sample fractions and the PTD results prove to be too impractical for use, an additional proportional analysis will be used to assist in eliminating the dependence on the difference of total numbers found with the following formula:

Where:  $(n_i/N) \times 100$

$n_i$  = number of taxa 'i' that the taxonomist counted

N = total count of all organisms in sample by the taxonomist

5. Corrective actions include determining problem areas (taxa) and consistent disagreements and addressing problems through taxonomist interactions. These actions help to rectify disagreements resulting from identification to a specific taxonomic level.

### 11.7.2.3 Taxonomic QC Review & Reconciliation

The Indicator QC Coordinator prepares a report or technical memorandum to quantify aspects of taxonomic precision, assess data acceptability, highlight taxonomic problem areas, and provide recommendations for improving precision. This report is submitted to the HQ Project Management Team, with copies sent to the primary and QC taxonomists. Another copy is maintained in the project file. Significant differences may result in the re-identification of samples by the primary taxonomist and a second QC check by the secondary taxonomist. Each laboratory prepares reference/voucher samples. These samples will be identified and digitally referenced (a photograph with taxonomic information

superimposed on the photograph and in the file name) and will be included in an electronic file folder on the NARS SharePoint Site.

All samples are stored at the laboratory until the Project Lead notifies the laboratory regarding disposition.

**Table 11-2 Laboratory quality control: zooplankton indicator.**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>IDENTIFICATION</b>			
<b>Independent identification by outside taxonomist</b>	All uncertain taxa	Uncertain identifications to be confirmed by expert in particular taxa	Record both tentative and independent IDs
<b>Use standard taxonomic references</b>	For all identifications	All keys and references used must be on bibliography prepared by another laboratory	If other references desired, obtain permission to use from Project Facilitator
<b>Prepare reference collection</b>	Each new taxon per laboratory	Complete reference collection to be maintained by each individual laboratory	Laboratory Manager periodically reviews data and reference collection to ensure reference collection is complete and identifications are accurate
<b>External QC</b>	10% of all samples completed per laboratory	Efficiency (PTD) $\geq$ 85%	If PTD < 85%, implement recommended corrective actions.
<b>DATA VALIDATION</b>			
<b>Taxonomic "reasonable-ness" checks</b>	All data sheets	Genera known to occur in given lake or geographic area	Second or third identification by expert in that taxon

## **12.0 RESEARCH INDICATOR: DISSOLVED GASES**

Carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O) concentrations in air and dissolved gases will be measured using gas chromatography at the USEPA's Office of Research and Development Laboratory in Cincinnati, Ohio. The relative abundance of the stable isotopes <sup>12</sup>C and <sup>13</sup>C in the dissolved CO<sub>2</sub> and CH<sub>4</sub> will be measured by colleagues at the University of Quebec. The laboratory procedures for these analysis will not be included in this manual, but are available upon request.

### **13.0 RESEARCH INDICATOR: FISH eDNA**

Information on this indicator is contained in other research documents.

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## **APPENDIX A: LABORATORY REMOTE EVALUATION AND VERIFICATION FORMS**



## Document Request Form – Chemistry Laboratories

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The USEPA and its state and tribal partners will conduct a survey of the nation's lakes, ponds, and reservoirs. This National Lakes Assessment is designed to provide statistically valid regional and national estimates of the condition of lakes. Consistent sampling and analytical procedures ensure that the results can be compared across the country.

As part of the National Lakes Assessment (NLA) 2017, 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 the USEPA's NLA.

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): analysis of Proficiency Testing samples and/or a site visit. All laboratories will need to complete the following form:

- A *signature* on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for chemistry laboratories conducting analyses for the NLA 2017.

In order for us to determine your ability to participate as a laboratory in the NLA, we are requesting that you submit the following documents (if available) for review:

- Documentation of a successful *quality assurance audit* from a prior survey that occurred within the last 5 years (if you need assistance with this please contact the individual indicated below)
- A copy of your Laboratory's *accreditations and certifications* if applicable (i.e. NELAC, ISO, state certifications, etc...)

If your laboratory can provide either documentation of a prior audit or accreditation, no other documentation is needed. If **neither** of the above is complete, please provide the following information.

- A copy of your Laboratory's *Quality Manual*
- Standard Operating Procedures (SOPs)* for your laboratory for each analysis to be performed (if not covered in NLA 2017 Laboratory Manual)
- Other documentation supporting your laboratory's ability to meet the required level of data quality (if available)

This documentation may be submitted electronically via e-mail to [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov). Questions concerning this request can be submitted to [pollard.amina@epa.gov](mailto:pollard.amina@epa.gov) (202-566-2369) or [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov) (202-564-0417).

## Laboratory Signature Form – Chemistry Laboratories

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I \_\_\_\_\_ certify that the \_\_\_\_\_ laboratory, located in \_\_\_\_\_, will abide by the following standards in performing **chemistry** data analysis and reporting for the National Lakes Assessment (NLA).

- 1.) Utilize procedures identified in the NLA 2017 Laboratory Operations Manual (or equivalent). If using equivalent procedures, please provide procedures manual.
- 2.) Read and abide by the NLA 2017 Quality Assurance Project Plan (QAPP) and related Standard Operating Procedures (SOPs).
- 3.) Have an organized IT system in place for recording sample tracking and analysis data.
- 4.) Provide data using the template provided in the Laboratory Operations Manual.
- 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, 2018 or as otherwise negotiated with the USEPA.
- 6.) Participate in a laboratory technical assessment or audit if requested by an USEPA NLA staff (this may be a conference call or on-site audit).

Signature \_\_\_\_\_ Date \_\_\_\_\_

## Document Request Form - **Biology** Laboratories

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The USEPA and its state and tribal partners will conduct a survey of the nation's lakes, ponds, and reservoirs. This National Lakes Assessment is designed to provide statistically valid regional and national estimates of the condition of lakes. Consistent sampling and analytical procedures ensure that the results can be compared across the country.

As part of the National Lakes Assessment (NLA) 2017, 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 **biology** analyses under this project. Our review will be assessing your laboratory's ability to receive, store, prepare, analyze, and report sample data generated under the USEPA's NLA 2017.

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

- A *signature* on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for chemistry laboratories conducting analyses for the NLA 2017.

In order for us to determine your ability to participate as a laboratory in the NLA, we are requesting that you submit the following documents (if available) for review:

- Documentation of a successful *quality assurance audit* from a prior survey that occurred within the last 5 years (if you need assistance with this please contact the individual listed below)
- A copy of your Laboratory's *accreditations and certifications* if applicable (i.e. NELAC, ISO, state certifications, etc...)

If your laboratory can provide either documentation of a prior audit or accreditation, no other documentation is needed. If **neither** of the above is complete, please provide the following information:

- Documentation of NABS *certification* for the *taxonomists* performing analyses (if available)
- A copy of your Laboratory's *Quality Manual*
- Standard Operating Procedures* (SOPs) for your laboratory for each analysis to be performed (if not covered in NLA 2017 Laboratory Manual)
- Other documentation supporting your laboratory's ability to meet the required level of data quality (if available)

This documentation may be submitted electronically via e-mail to [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov). Questions concerning this request can be submitted to [pollard.amina@epa.gov](mailto:pollard.amina@epa.gov) (202-566-2369) or [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov) (202-564-0417)

## Laboratory Signature Form – Biology Laboratories

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I \_\_\_\_\_ certify that the \_\_\_\_\_ laboratory, located in \_\_\_\_\_, will abide by the following standards in performing **biology** data analysis and reporting for the National Lakes Assessment (NLA).

- 1.) Utilize procedures identified in the NLA 2017 Laboratory Operations Manual (or equivalent). If using equivalent procedures, please provide procedures manual.
- 2.) Read and abide by the NLA 2017 Quality Assurance Project Plan (QAPP) and related Standard Operating Procedures (SOPs).
- 3.) Have an organized IT system in place for recording sample tracking and analysis data.
- 4.) Use taxonomic standards outlined in the NLA 2017 Laboratory Manual.
- 5.) Participate in taxonomic reconciliation exercises during the field and data analysis season, which include conference calls and other laboratory reviews.
- 6.) Provide data using the template provided in the Laboratory Operations Manual.
- 7.) 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, 2018 or as otherwise negotiated with the USEPA.
- 8.) Participate in a laboratory technical assessment or audit if requested by USEPA NLA staff (this may be a conference call or on-site audit).

Signature \_\_\_\_\_ Date \_\_\_\_\_

## **APPENDIX B: SAMPLE LABORATORY FORMS**



### Benthic Macroinvertebrate Laboratory Bench Sheet

Project Name/Number \_\_\_\_\_ Serial ID \_\_\_\_\_  
 Waterbody Name \_\_\_\_\_ Site ID \_\_\_\_\_  
 Sorter (initially spread sample) \_\_\_\_\_ Sort Date \_\_\_\_\_ Collection Date \_\_\_\_\_

Grid Order	Sorter's Initials	Random Number Grid ID	Number of Individuals per Grid	Cumulative Number of Organisms
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
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21				
22				
23				
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25				
26				
27				
28				
29				
30				





### Zooplankton Enumeration Data Sheet

Sample # \_\_\_\_\_ Lake \_\_\_\_\_ Laboratory # \_\_\_\_\_

Date Collected \_\_\_\_\_ Depth of tow \_\_\_\_\_ Analyzed by \_\_\_\_\_

Working Volume (mL) \_\_\_\_\_ Milliliters in subsample (rotifers) \_\_\_\_\_ Split \_\_\_\_\_

↓ Taxa / Count →	A	B	C	D
<b>Total Mature Copepoda</b>				
<b>Total Immature Copepoda</b>				
<b>Total Cladocera</b>				
<b>Total Rotifera</b>				
<b>Total Other Organisms</b>				

Note: For Rotifers only A and B counts are made.





## **APPENDIX C: STATE SAMPLE TRACKING SPREADSHEET**

Provided on the NARS SharePoint site or from the Laboratory Review Coordinator.

## **APPENDIX D: REPORTING TEMPLATES**

Templates will be provided on the NARS SharePoint Site.

## **APPENDIX E: SUPPORTING METHODS**

As needed.