United States Environmental Protection Agency
Office of Water
Washington, DC
EPA 841-B-11-004

2012 National Lakes Assessment

Laboratory Operations Manual

Version 1.1 October 9, 2012

NOTICE

The intention of the 2012 National Lakes Assessment (NLA 2012) 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:

2012 National Lakes Assessment: Quality Assurance Project Plan (EPA 841-B-11-006)

2012 National Lakes Assessment: Site Evaluation Guidelines (EPA 841-B-11-005)

2012 National Lakes Assessment: Field Operations Manual (EPA 841-B-11-003)

This document (Laboratory Operations Manual) contains information on the methods for analyses of the samples for ten indicators (algal toxins (microcystins), benthic macroinvertebrates, phytoplankton, sediment dating, sediment diatoms, sediment mercury, triazine pesticide screen, water chemistry and chlorophyll A, and zooplankton) to be collected during the project, quality assurance objectives, sample handling, and data reporting. (Dissolved carbon method will not be included in this laboratory operations manual, as explained in this manual.) These 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 2012. 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.

The suggested citation for this document is:

U.S. EPA. 2012. 2012 National Lakes Assessment. Laboratory Operations Manual. EPA-841-B-11-004. U.S. Environmental Protection Agency, Washington, DC.

NOTICE

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LIST OF ACRONYMS

ANC	acid neutralizing capacity
CO_2	carbon dioxide
CPR	cardiopulmonary resuscitation
DI	deionized
DO	dissolved oxygen
DOC	dissolved organic carbon
EMAP	Environmental Monitoring and Assessment Program
EPA	Environmental Protection Agency
ETOH	ethyl alcohol
FOM	Field Operations Manual
GIS	geographic information system
GPS	global positioning device
HDPE	high density polyethylene
H_2S	hydrogen sulfide
LOM	Lab Operations Manual
MPCA	Minnesota Pollution Control Agency
NALMS	North American Lakes Management Society
NH_4	ammonium
NIST	National Institute of Standards
NO_3	nitrate
OSHA	Occupational Safety and Health Administration
PCB	polychlorinated biphenyl
PHab	physical habitat
PDE	percent difference in enumeration
PSE	percent sorting efficiency
PTD	percent taxonomic disagreement
QA	quality assurance
QAPP	Quality Assurance Project Plan
QA/QC	quality assurance/quality control
QCCS	quality control check solution
QRG	Quick Reference Guide
RMSE	root mean square error
RPD	relative percent difference
SEG	Site Evaluation Guidelines
SOPs	Standard Operating Procedures
TN	total nitrogen
TOC	total organic carbon
TP	total phosphorus
TSS	total suspended solids
TVS	total volatile solids
USGS	United States Geological Survey

2012 NATIONAL LAKES ASSESSMENT LABORATORY OPERATIONS MANUAL

The U.S. Environmental Protection Agency (EPA), in partnership with state and tribal organizations, has designed the 2012 National Lakes Assessment (NLA) to assess the condition of the nation's lakes, ponds and reservoirs (referred to collectively as lakes throughout the document). 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 the Nation's waters. In addition to lakes, the NARS will assess coastal waters, wetlands, rivers, and streams in a revolving sequence.

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

1) document the standardized sample processing and analysis procedures used in the various laboratories for the NLA 2012
2) provide guidance for data quality and a performance-based method approach to obtain comparable results across all participating laboratories.

Detailed laboratory procedures are described for the following indicators: algal toxins (microcystins), benthic macroinvertebrates, phytoplankton, sediment dating, sediment diatoms, triazine pesticide screen, water chemistry and chlorophyll A, and zooplankton. A couple of indicators are research indicators and will be completed in collaboration with USGS, these include: sediment mercury and dissolved carbon. It should be noted that specific laboratory analysis procedures for water chemistry samples are not presented here. Procedures used at the national laboratory (EPA ORD Corvallis) are available as a separate document upon request. 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 technical director (EPA Office of Water).

1 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 lab should follow its institutional or organizational requirements for instrument maintenance. Specific lab qualification documentation required for analysis is contained in the Quality Assurance Project Plan (QAPP).

1.2 Roles and Contact Information

The **EPA 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 **NARS Information Management (IM) Coordinator** tracks the location of each NLA 2012 sample that involves post-processing. The coordinator will be the labs main point of contact in regards to sample tracking and data submission.

Table 1.1 Contact information

Title	Name	Contact Information
EPA HQ Project Lead	Amina Pollard, OW	pollard.amina@epa.gov 202-566-2360
EPA HQ Project QA Lead	Sarah Lehmann, OW	lehmann.sarah@epa.gov 202-566-1379
EPA HQ Logistics Lead	Marsha Landis, OW	landis.marsha@epa.gov 202-564-2858
Information Management Center Coordinator	Marlys Cappaert, SRA International Inc.	cappaert.marlys@epa.gov 541-754-4467 541-754-4799 (fax)

1.3 Sample Tracking

Samples are collected by a large number of different field crews during the index period (May through September). The actual number of lakes sampled on a given day will vary widely during this time. Field crews will submit electronic forms when they have shipped samples and the NARS IM Center will input each sample into the NARS IM database. Laboratories can track sample shipment from field crews by accessing the NARS IM database. Participating laboratories will be given access to the NARS IM system, where they can acquire tracking numbers and information on samples that have been shipped to them by field crews (either by overnight shipment for perishable samples or batch shipments for preserved samples). Upon sample receipt, the laboratory must immediately log in to the database and confirm that samples have arrived. Overnight samples may not be loaded into the database prior to sample arrival, but should be tracked by the laboratory and receipt information inputted into the database when sample information is loaded. Each lab will make arrangements with the NARS IM Coordinator, listed above, to ensure access is granted.

When the samples arrive from the field crews, laboratories should also receive tracking forms in the shipment (refer to the NLA 2012 FOM). These forms will list the samples that should be included in the shipment. Laboratory personnel should cross check the forms with the samples received to verify that there are not any inconsistencies. If any sample is missing or damaged, contact the NARS IM Coordinator immediately.

1.4 Reporting

All labs must provide data analysis information to the HQ Project Management Team and the NARS IM Center by March 30, 2013 or earlier as stipulated in contractual agreements. These reports must include the following information:

- Sample Type (indicator)
- Site ID (ex: NLA12_AL-107)
- Sample ID (ex: 999000)
- Pertinent information to the indicator
- Metadata for all fields

See Appendix D for reporting templates that labs will submit electronically.

The submitted file name must state the following:

- Indicator name (ex: microcystin)
- Date of files submission to NARS IM Center by year, month, and day (ex: 2011_11_01)
- Lab name (ex: MyLab)

Combined, the file name would look as follows: Microcycstin_2011_11_01_MyLab.xlsx

As specified in the QAPP, remaining sample material and specimens must be maintained by the EPA's designated laboratory or facilities as directed by the NLA 2012 Project Lead. All samples and raw data files (including logbooks, bench sheets, and instrument tracings) are to be retained by the laboratory for 3 years or until authorized for disposal, in writing, by the EPA Project Leader. Deliverables from contractors and cooperators, including raw data, are permanent as per EPA Record Schedule 258. EPA's project records are scheduled 501 and are also permanent.

2 LAB QUALITY CONTROL

As part of the NLA 2012 field samples will be collected at each assessment site. These samples will be sent to laboratories cooperating in the assessment. To ensure quality, each Project Cooperator laboratory analyzing samples from the NLA 2012 will receive an evaluation from an NLA Lab Evaluator. All Project Cooperator laboratories will follow these guidelines.

No national program of accreditation for lab processing for most of our indicators currently exists. For this reason, a rigorous program of laboratory evaluation has been developed to support the 2012 NLA.

Given the large number of labs participating in the NLA 2012, it is not feasible to perform an assistance visit[a] (AV) on each of these laboratories. An AV would include an on-site visit to the lab lasting at least a day. As a result, the EPA Headquarters Project Management Team will conduct remote review of lab certifications and accreditations of all labs and an inter-laboratory comparison will be performed between some labs (mainly for biological indicators). If issues arise from the remote review or inter-laboratory comparison that cannot be resolved remotely then an on-site visit to the lab will be performed. The NLA 2012 Project Management Team believes this approach meets the needs of this assessment and can ensure quality control on data generated by the participating labs.

2.1 Remote Evaluation/Technical Assessment

Procedural review and assistance personnel are trained to the specific implementation and data collection methods detailed in this 2012 NLA LOM. Laboratory evaluation reinforces the specific techniques and procedures for both field and laboratory applications. A remote evaluation procedure has been developed for performing assessment of all labs.

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

The procedure being utilized involves requesting the laboratory to provide documentation of its policies and procedures. For the 2012 NLA project, we have requested that each participating laboratory provide the following documentation:

- The laboratory's Quality Manual, Quality Management Plan or similar document
- Standard Operating Procedures (SOPs) for each analysis to be performed
- Long term Method Detection Limits (MDLs) for each instrument used and Demonstration of Capability (DOC) for each analysis to be performed
- A list of the laboratory's accreditations and certifications, if any
- Results from Proficiency Tests for each analyte to be analyzed under the NLA project

If a laboratory has clearly documented procedures for sample receiving, storage, preservation, preparation, analysis, and data reporting; has successfully analyzed Proficiency Test samples (if required by EPA, EPA will provide the PT samples); has a Quality Manual that thoroughly addresses laboratory quality including standard and sample preparation, record keeping and QA non-conformance;

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

participates in a nationally recognized or state certification program; and has demonstrated ability to perform the testing for which program/project the audit is intended, then the length of an on-site visit will be minimum, if not waived entirely. A final decision on the need for an actual on-site visit should be made after the review and evaluation of the documentation requested.

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

In addition, all labs must sign a Lab Signature Form (in **APPENDIX B: LABORATORY REMOTE EVALUATION FORMS**) indicating that they will abide by the following:

1. Utilize procedures identified in the 2012 NLA Lab Operations Manual (or equivalent). If using equivalent procedures, please provide procedures manual to demonstrate ability to meet the required MQOs.
2. Read and abide by the 2012 NLA 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 Lab 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, 2013 or as otherwise negotiated with EPA.
6. Participate in a lab technical assessment or audit if requested by EPA NLA staff (this may be a conference call or on-site audit).

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

1. Use taxonomic standards outlined in the 2012 NLA Lab Manual.
2. Participate in taxonomic reconciliation exercises during the field and data analysis season, which include conference calls and other lab reviews (see more below on Inter-laboratory comparison).

2.1.1 Water Chemistry Laboratories

The water chemistry portion of this process has been developed and is being coordinated by the Quality Assurance Team from the US EPA Region 3 Environmental Assessment and Innovations Division, Office of Analytical Services and Quality Assurance, Technical Services Branch. This procedure is deemed appropriate because many laboratories participate in one or more national laboratory accreditation programs such as the National Environmental Laboratory Accreditation Program (NELAP), International Organization for Standardization (ISO-17025) as well as various state certification programs which include strict requirements around documentation and procedures as well as site visits by the accrediting authority. The laboratories that were selected for the NLA 2012 meet these qualifications and as such have demonstrated their ability to function independently. This process is one that has been utilized in Region 3 for many years and is designed around the national accrediting programs described above.

2.2 Inter-laboratory Comparison

An inter-laboratory investigation is being implemented for the labs performing analysis on benthic macroinvertebrates, phytoplankton, sediment diatoms, and zooplankton data for the 2012 NLA. This

process is defined as an inter-laboratory comparison since the same protocols and method will be used by both laboratories as described in this manual. No site visit is envisioned for these labs unless the data submitted and reviewed by EPA does not meet the requirements of the inter-laboratory comparison described.

2.3 Assistance Visits

Assistance Visits will be used to:

- Confirm the NLA 2012 Lab Operations Manual (LOM) methods are being properly implemented by cooperator laboratories.
- Assist with questions from lab personnel.
- Suggest corrections if any errors are made in implementing the lab methods.

Evaluation of the labs will take the form of administration of checklists which have been developed from the LOM to ensure that labs are following the methods and protocols outlined therein. The checklist will be administered on-site by a qualified EPA scientist or contractor.

3 ALGAL TOXIN (MICROCYSTIN) METHODS

This method, as adapted from *Microtiter Plate Enzyme-Linked Immuno-Sorbent Assay for Microcystin* (Loftin 2006). Results are for water samples and concentrations are reported between 0.10µg/L and 5.0µg/L without dilution, where the detection limit is 0.1µg/L and the reporting limit is 0.15µg/L. Samples with concentrations >5.0 µg/L require dilution and re-analysis. Non-detects are reported as "<0.10 µg/L."This method is suitable for water and algae samples that have been lysed and/or filtered. This SOP is based on the use of an immunoassay kit manufactured by Abraxis. Samples may be held for no longer than 14 days at 4 degrees C (but are being shipped to the batching lab in one week intervals) and for several months if frozen.

Algal toxin (microcystin) samples will be held on ice by field crews and shipped from field crews to a contract batching lab. The contract batching lab will freeze the samples and send the batched samples to the analysis lab on a regular basis during the project as collection of the field samples is completed to avoid delays in processing and identifying samples. The samples will arrive at the analysis lab and can be held in the freezer for several months, though algal toxin analysis labs 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.

3.1 Responsibility and Personnel Qualifications

This procedure may be used by any person who has received training in processing and/or identification of algal toxin samples. It is also important that the analyst maintains contact with other algal toxin experts through professional societies and other interactions, and keeps up with the pertinent literature, since analysis methods change over time. Precautions

This SOP is to be used in conjunction with a lab approved Chemical Hygiene Plan. Also, consult the Chemical Hygiene Plan for information on and use of all personal protective equipment (PPE).

The Stopping Solution consists of a weak acid. Do not allow it get on your clothes or yourself. Wash the acid off immediately with copious amount of water.

3.2 Equipment/Materials

Descriptions of equipment to use are listed below.

Adhesive Sealing Film (Parafilm) for Micro Plates (such as Rainin, non-sterile, Cat. No. 96-SP-100): Used to cover plates during incubation.

Data Template

Distilled Deionized Water: For diluting samples.

Immunoassay Quality Assurance Sheet: This is used by the QA checker and will be written on when results are printed off.

2 glass scintillation vials (20 mL)

Microcystins Plate Kit (Abraxis)

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)

Orbital Shaker Table (such as American Shaker Table V, Model R4140): To be used for mixing microtiter plates during incubations.

Paper Towels: For blotting the microtiter plates dry after washing.

Permanent Marker (Sharpie Fine Point): For labeling samples, bottles, plates and covers.

Pipette (100 µL) and Tips: For measuring and transferring standards, controls, and samples into the antibody coated plates.

Pipette (1000 µL) and Tips: For diluting samples for reruns.

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)

3.3 Procedure

3.3.1 Sample Preparation

1. Before beginning analysis, samples must be run through a freeze thaw procedure three times. Samples will be frozen by the batching lab prior to shipping. Lab recipient must follow the chain-of-custody procedures for the NLA 2012.
2. For the first freeze-thaw cycle, thaw (at room temperature) the 500 mL bottles the samples were collected in. Aliquot 10 mL of each well mixed sample into the new, labeled 20 mL glass scintillation vial, one per sample. Place the 20 mL scintillation vial in a freezer to complete the two additional freeze-thaw cycles (for a total of three). All thaw cycles should be completed at room temperature.
3. After the last freeze-thaw cycle, filter approximately 10 mL or each sample 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. Allow immunoassay kits and samples to warm to room temperature before analyzing (approximately 1 hour). Make certain there is enough of all the reagents to complete the number of analyses before beginning. If not, allow another kit to warm.
5. Assemble the sample bottles, ensuring that samples are separated by project and include a QC sample for each project. For each set of 10 samples, make the first and fifth samples lab duplicate samples and the tenth sample a lab spiked duplicate. Record the sample ID, QC sample ID, date analyzed and project code in the algal toxin central database.
6. Using the Softmax software (or other software if appropriate), enter into the template the location of the Standards, Controls and Samples on the microtiter plate (example **Figure 3.1**).

Templates can contain rows, labeled with a marking pen, of strips of 8 wells that snap into the blank frame.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S3	U4	P10	U16	U21	U27	U32	U39	U44	P50	U56
B	S2	S4	U5	C3	L16	L21	U28	U33	U40	U45	C7	L56
C	S3	S5	U6	U11	U17	U22	U29	U34	P40	U46	U511	U57
D	S4	C2	L6	L11	U180	U23	U30	U35	C6	L46	L51	U58
E	S5	U1	U7	U12	U19	U24	P30	U36	U41	U47	U52	U59
F	C1	L1	U8	U13	U20	U25	C5	L36	L41	U48	U53	QC3
G	S1	U2	U9	U14	P20	U26	U31	U37	U42	U49	U54	P59
H	S2	U3	U10	U15	C4	L26	L31	U38	U43	U50	U55	C8

Figure 3.1 Example microcystin template

[Key: S = standard; C = 0.75 µg/L control – supplied with ELISA kit; QC = quality control; U = unknown (sample); L = unknown duplicate (sample); P = spiked duplicate unknown (sample)]

7. Analyze all 5 standards (0.00, 0.15, 0.40, 1.00 and 5.00) in duplicate. Space the sets of standards at the beginning. Prepare the appropriate template and print it for reference when loading the standards and samples. At this time, enter plate name, file name, and operator into the standard sample sheet and save all information.
8. Turn the plate reader on so it can warm up. The plate reader needs a minimum of 30 minutes to warm up. The plate reader may need to be turned on before the computer boots up so that the computer can control and access the plate reader.

3.3.2 Analysis Procedure

1. Analysis methods described in this manual are in agreement with the manufacturer's instructions. These can also be found in the kit contents.
2. Prepare spiked samples by adding 15 µL of a 25 µg/L microcystin-LR standard solution to 500 µL of sample in a labeled LC vial. Cap and vortex. (Note on 25 µg/L microcystin-LR standard solution: This solution does not need to be made fresh daily. Stock m microcystin-LR standard can be made by dilution in LC/MS grade methanol. Be sure to check purity by UV-VIS extinction coefficient or LC/MS/MS. It is acceptable to make batches from the stock in glass 2 mL LC vials and freeze them half-full. The final methanol content is 5% or less in the 25 µg/L MCLR standard used for ELISA.)
3. Using the 100-µL pipette, add 50 µL of the standards, controls, samples and spiked samples (prepared earlier) to the appropriate wells in the plate.
4. Add 50 µL of the pink antibody solution to each well using the multi-channel pipette and a reagent reservoir. Place the sealing Parafilm over the wells. Place the plate on the orbital shaker table. Protect tray from light, and set the speed for 180 rpm and the timer for an hour and a half. After 90 minutes, carefully remove the Parafilm.
5. Empty the plate into the sink, pat dry with a stack of paper towels, and then wash the wells of the plate three times with 250 µL of washing solution using the multi-channel pipette. Each time you add the washing solution, let the washing solution set about 45 seconds and then empty into the sink and use the paper towels as before.
6. Add 100 µL of enzyme conjugate solution to all wells using the multi-channel pipettor.

7. Cover the wells with Parafilm and place on the orbital shaker table in a dark corner; set shaker to 180-rpm for 30 min.
8. After 30 minutes, remove the Parafilm and rinse the wells three times again with 250 µL of washing solution as described in step 4.
9. Add 100 µL of substrate solution to the wells using the multi-channel pipette and reagent reservoir. This color solution will make the contents have a blue hue. Cover with Parafilm and protect from light. Incubate on the orbital shaker at 180 rpm for 25 min.
10. Remove the Parafilm and add 50 µL of stopping solution to the wells. This will turn the contents a bright yellow color. After you have added the stopping solution, you must read the plate within 15 minutes using the plate reader.

3.3.2.1 Reading the Plate

1. A plate reader and appropriate software are used for controlling the microtiter plate reader and for calculating results. Ensure that the setting in the plate reader software (e.g. PC Mate) program agrees with the manufacturer's (Abraxis) instructions. The current setting from Abraxis are:
    a. Wave length: 450 nm
    b. Curve: Semi-log
    c. Display: Analyzed
    d. Endpoint: L1, Auto mix: on, cal: on, disk: on, print: OFF
2. The software calculates the values of the two samples from the calibration curve and averages the results to a standard curve. The standard curve should have a correlation coefficient of .99. The absorbency of the blank must be standard correlation coefficient >1.400. Samples with concentrations >5.0 µg/L require dilution and re-analysis. Non-detects are reported as "<0.10 µg/L."
3. The lab will use a data entry sheet to record information pertaining to the entire plate's samples such as the Lab ID, project code, concentrations to report, analysis data, remarks, and the lab technician's initials. The entire plate's samples go on a single data entry sheet.
4. When recording the concentration of the sample, the mean concentration, the last of the three printed out values, will be used. The strip will show if it is "HI" for out of range values. Samples that need dilution because of "HI" concentrations need approval from the lab supervisor and should be re-done in the next round. The same is true for any other re-do, such as if the duplicates do not agree, stop solution runs out, or a bad standard curve results. Non-detects (less than 0.10 µg/L) are flagged as "nd." QC samples go in a separate database. Print out all sheets.
5. Dispose of solution in plates in a lab sink. Rinse plates and sink with water to dilute the weak acid present.

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

1. 1. 1:10 dilution
    a. Pipette 100µl from the sample in to the tube.
    b. Add 900 µl of distilled water to sample above. (Note: Dilutions may also be made using the kit's dilutenet rather than distilled water.)
    c. Mix by Vortexing.
    d. Multiply final concentration by 10.
2. 1:100 dilution

a. Pipette 10µl from the sample.
b. Add 990µl of distilled water to sample. (Note: Dilutions may also be made using the kit's dilutenet rather than distilled water.)
c. Mix by Vortexing.
d. Multiply final concentration by 100.

3. Other dilutions can be calculated if needed.

3.4 Pertinent QA/QC Procedures

1. Before finishing with the data entry sheet, check control measurements (supplied with the ABRAXIS kit). To determine if the control is acceptable, type in the found value in the *Analyzed* column, this is in a blue font at the bottom of the page. It will calculate a percent difference, which must be within 20% of the control concentration.
2. Precision and accuracy should both be ±20 percent.
3. Prepare a Quality Assurance Sheet for each project with information from the data entry sheet. Do a bottle check (a verification of the log-in accuracy) on the samples analyzed. Compare the information printed in the lab's Log-In binder to the bottle and look for discrepancies. Report any discrepancies to the Project Manager and correct them in the binder. As noted above, the applicable software calculates the values of the samples from the Calibration Curve and averages the two results to a standard curve. The standard curve should have a correlation coefficient of .99. The absorbency of the blank must be standard correlation coefficient >1.400.
4. Laboratory duplicates should have a percent Relative Standard Deviation (%RSD) of 28.3 percent or less when compared to each other (as suggested by the ELISA kit manufacturer). If duplicates are outside this range, then they are re-analyzed in the next run.
5. The theoretical total concentration of the laboratory spiked duplicates should be 0.75 µg/L plus the concentration of the un-spiked sample. Laboratory Spiked Duplicates must have an actual value of +/-20 percent of the theoretical concentration of the spiked sample. If spiked samples occur outside this range, then the sample and the laboratory spiked sample are re-analyzed.
6. "A" designated archived project sample is re-analyzed with every run set. Maintain a control chart of the running historical average of the concentration from each run for these samples. The concentration of the QC sample for each successive run must be ± 20% of the historical average to be acceptable.

Table 3.3 Sample analysis quality control activities: microcystin indicator quality control activity

Quality Control Activity	Description and Requirements	Corrective Action
Laboratory Duplicate	Every first and fifth sample are duplicate samples analyzed for QC purposes.	Samples are re-analyzed if samples do not agree or bad standard deviation curves
Laboratory Spiked Sample	Every tenth sample analyzed is a laboratory spiked duplicate sample that contains known microcystin concentrations	Samples are re-analyzed if samples do not agree or bad standard deviation curves
Identical Sample	Identical sample designated by a letter S attached to the log number. Final concentration will be 0.75 µg/L of Microcystin-LR plus the ambient concentration	Samples are re-analyzed if samples do not agree or bad standard deviation curves
Project Quality Control Sample	Designated project archive sample is re-analyzed with every run set for the project. Control charts are maintained for these samples.	Samples are re-analyzed if samples do not agree or bad standard deviation curves

4 BENTHIC MACROINVERTEBRATE METHODS

This procedure is adapted from *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 EtOH and shipped from field crews to a contract batching lab. The contract batching lab will send the batched samples to the analysis lab. Preserved samples will arrive in the analysis lab and can be held for several months. Benthic invertebrate analysis labs 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.

4.1 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.

4.2 Precautions

4.2.1 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 by senior biology staff familiar with processing benthic samples must perform a QC check. Only qualified personnel (QC Officers) will perform the QC checks in the Pertinent QA and QC Procedures section. The 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.

4.2.2 Taxonomy Precautions

Base all the identifications on current published taxonomic references.

If technical literature citations specifying nomenclatural validity are not available or otherwise are unknown, use taxon names from the Integrated Taxonomic Information System (ITIS), available on the Web at: http://www.itis.usda.gov/.

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).

4.3 Equipment/Materials

4.3.1 Sorting and Subsampling Equipment/Materials

U.S. 35 sieve (500 µm)
Round buckets
Standardized gridded screen (370-µm)
Mesh screen, 30 squares (6 cm^2 each) with white plastic holding tray[1]
6-cm scoop
6-cm^2 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
Benthic Sample Log-In Form
Benthic Macroinvertebrate Laboratory Bench Sheet (Appendix A)
Stereo zoom microscope (6-10X magnification)

4.3.2 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
70 - 80% denatured ethanol in plastic wash bottle
Benthic Macroinvertebrate Taxonomic Bench Sheet
Hand tally counter

4.4 Procedure

4.4.1 General

[1]Some laboratories may choose not to use the gridded screen in a plastic holding tray.

1. Record receipt of samples in the laboratory on the Benthic Sample Log-In form (**APPENDIX C: 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% ethanol if necessary. After refilling the sample containers, store them until sorting begins.
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% ethanol.
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 C: 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)

4.4.2 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 station name, station location, station number, project name, bench number, sample type, date the sample was collected, and the field team who collected the sample (e.g., Team 1). 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 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 (if 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 lab, before subsampling has begun on that particular sample. Magdych (1981, Hydrobiologia 85(2): 157-159) 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 µm). 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 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 4.4.3**, #1). 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.

4.4.3 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, 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, then stop sorting and preserve the remaining unsorted material in 70-80% denatured ethanal, and store remaining unsorted material 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 ethanol.
7. Keep a rough count of the number of organisms removed and enter the number of organisms found in each grid under that 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[2] 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.

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

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 QC Officer will count any missed organisms found and place them into the sample vial, or other suitable sample vial. The 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 4.4.2** #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% ethanol). 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 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 QC Officer and the Project Manager.
16. Record the sorting date each sample was completed near the top right corner of the bench sheet.

4.4.4 Taxonomy Procedures

1. The taxonomic target for benthic invertebrates is identified in **Section 4.4.4.1**.
2. Upon receipt of a set of sample vials from the project cooperator or contractor laboratory, remove the chain-of-custody 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 chain-of-custody 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 ethanol 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% ethanol). Identify organisms to the correct taxonomic level for the project (usually genus, Attachment 4). 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. Also enter the Taxonomic Serial Number (TSN; found in ITIS). Use the following steps to compare the final taxa list for each site to that of the ITIS website (http://www.itis.usda.gov). Record the TSN from ITIS on the Electronic Bench Sheet (TBD).
    a. Copy block of taxa names to a text file.
    b. Save the text file.
    c. Go to the ITIS taxa match screen (http://www.itis.usda.gov/taxmatch_ftp.html).
    d. Follow the onscreen instructions to upload the file. Use all of the current defaults.
    e. Finish with two lists, one of matches with TSNs and one with non-matches. Check the non-matches for the following common problems.
        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
    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 Project Facilitator will coordinate any necessary inter-lab 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 ethanol, and cap tightly.
13. Re-package the samples and slide-mounted specimens carefully, and sign and date the chain-of-custody 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 in an envelope to the Project Facilitator. Ship samples and bench sheets separately.

4.4.4.1 Taxonomic Level of Effort

This is the Standard Taxonomic Effort list for benthic macroinvertebrates (**Table 4.4.1**). 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 doddsl*). If the minimum taxonomic level cannot be achieved due to immature, damaged, or pupal specimens this should be noted in the data file "flag" variable (e.g., IM = y, DD = y, PP = y). If a unique taxon is determined for which the appropriate taxonomic level is not available in the literature and there are other taxa in that taxonomic level, these specimens shall be given a code of UN = y (e.g., Ephemerellidae *Drunella doddsi* and *Drunella* sp. UN = y vs. *Drunella* sp. UN = n) so that these specimens can be distinguished from specimens that are NOT unique and are to be grouped at a higher taxonomic level due to imprecise identification.

Table 4.1 Required level of taxonomic identification for benthic macroinvertebrates

Phylum	Class			Required Taxonomic Identification	Notes
ANNELIDA					
	Branchiobdellida			Family	
	Hirudinea			Genus	
	Oligochaeta			Genus	
	Polychaeta			Family	

ARTHROPODA					
	Arachnoidea				
		Acari		Genus	
	Insecta				
		Coleoptera		Genus	
		Diptera	**Except in the following cases:**	Genus	
			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	
COELENTERATA					
MOLLUSCA					
	Bivalvia			Genus	
	Gastropoda		**Except in the following case:**	Genus	
			Hydrobiidae	Family	
NEMERTEA				Genus	

4.5 Pertinent QA/QC Procedures

4.5.1 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 QC Officer will calculate PSE for each sample as follows:

Equation 4.1 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 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 to continue to monitor their sorting efficiency. However, if he or she shows marked improvement in sorting efficiency prior to completion of the next five samples, achieving ≥ 90% sorting efficiency, the 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, a 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.

4.5.2 Taxonomic QC

4.5.2.1 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.

4.5.2.2 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 lab send those samples to a QC taxonomist (another experienced taxonomist who did not participate in the original identifications). The original lab will complete a chain-of-custody 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.
3. 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 4.2 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 4.3 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.

4. The recommendation for PDE is 5% or less.
5. 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 are investigated and logged for indication of error patterns or trends.
6. 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.

4.5.2.3 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 Sharefile.

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

Table 4.2 Laboratory quality control: benthic indicator.

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
SAMPLE PROCESSING (PICK AND SORT)			
Sample residuals examined by different analyst within lab	10% of all samples completed per analyst	Efficiency of picking ≥ 90%	If < 90%, examine all residuals of samples by that analyst and retrain analyst
IDENTIFICATION			
Sorted samples re-identified by different analyst within lab	10% of all samples	Accuracy of contractor laboratory picking and identification ≥ 90%	If picking accuracy < 90%, all samples in batch will be reanalyzed by contractor
Independent identification by outside taxonomist	All uncertain taxa	Uncertain identifications to be confirmed by expert in particular taxa	Record both tentative and independent IDs
Use standard taxonomic references	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

Prepare reference collection	Each new taxon per laboratory	Complete reference collection to be maintained by each individual laboratory	Benthic Lab Manager periodically reviews data and reference collection to ensure reference collection is complete and identifications are accurate
External QC	10% of all samples completed per laboratory	PDE ≤ 5% PTD ≥ 85%	If PDE > 5%, implement recommended corrective actions. If PTD < 85%, implement recommended corrective actions.
DATA VALIDATION			
Taxonomic "reasonable-ness" checks	All data sheets	Genera known to occur in given lakes or geographic area	Second or third identification by expert in that taxon

5 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 lab. The contract batching lab will send the batched samples to the analysis lab. Preserved samples will arrive in the analysis lab and can be held for several months. Phytoplankton analysis labs 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.

5.1 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.

5.2 Precautions

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

5.3 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

5.4 Procedure

5.4.1 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.

5.4.2 Choose Count Method

5.4.2.1 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 [definition below] 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.

5.4.2.2 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).

5.4.3 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, 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.

5.4.4 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 µm).

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.

5.4.5 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).

5.5 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 (μm^3). Phytoplankton abundance (cells/mL) is calculated as follows:

Equation 5.1 Phytoplankton abundance.

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

where *count* = number of cells counted, *chamber* = chamber area (in mm^2), *numfields* = number of microscope fields, *field* = microscope field area (in 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 **Phytoplankton Measurement Data Sheet**. Submit the file electronically to EPA**.**

5.6 Pertinent QA/QC Procedures

5.6.1 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 ≥ 90% identification as determined by QC checks, previous samples will be re-counted and identified.

5.6.2 External Taxonomic QC

On 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 lab to send those samples to a QC taxonomist, a second experienced taxonomist who did not participate in the original identifications. The original lab will complete a chain-of-custody form and send it with the samples.

5.6.2.1 Plankton Re-identification

Duplicate processing (duplicate the processing steps presented in **Section 5.4.1 – 5.4.5**).

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 lab 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 5.2 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.

5.6.3 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. Soft-bodied algal samples are placed in glass containers with appropriate preservative (Lugol's). 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 Sharefile.

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

Table 5.1 Laboratory quality control: phytoplankton indicator.

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

6 SEDIMENT DATING METHODS

This method describes a procedure to determine the activity of Lead-210 (^{210}Pb) in sediment from lakes and ponds, which provides a relative estimate of the time of deposition of the bottom slice of sediment core samples. Decay of uranium in the earth's crust releases the gas radon. This gas produces 210Pb by decay in the atmosphere. The lead isotope enters the water through precipitation. In the water phase 210Pb is adsorbed to particulate matter and together they are deposited in the sediment of lakes and ponds. 2012Pb decays with a half life of approximately 22 years. The remaining amount of 210Pb in these sediment samples will reveal its relative age. Owing to the 22-year half life, this 2012Pb method covers the past period of 75-100 years. The procedures listed below are taken directly from ESS RAD Method 400 from Wisconsin State Laboratory of Hygiene.

Sediment dating samples will be shipped on ice from field crews to a contract batching lab. The contract batching lab will freeze the samples send the batched samples to the analysis lab. Samples will arrive in the analysis lab frozen and can be held for several months. Sediment dating analysis labs 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.

Sections of sediment cores are freeze dried and homogenized. The finely divided samples are transferred into 1 oz labeled slip cover cans (ointment cans). The cans are sealed with an epoxy and stored for one month to allow for the ingrowth of radon progeny. At the end of the ingrowth period the cans are counted on an n-type planar germanium detector.

Sediment dating results are flagged if some part of the sample collection, holding time, processing, or shipment is compromised and did not meet the requirements of the LOM for the NLA 2012.

6.1 Responsibility and Personnel Qualifications

All laboratory personnel shall be trained in advance in the use of equipment and procedures used during this standard operating procedure (SOP). All personnel shall be responsible for complying with all of the QA/QC requirements that pertain to this indicator.

6.2 Precautions

All personnel are responsible for being aware of proper health and safety precautions and emergency procedures.

6.3 Equipment/Materials

Ointment cans

10 ton epoxy

Disposable 5mL plastic mixing containers

Plastic stirrers

Materials to measure bulk density

Materials to freeze dry sediment samples

6.4 Procedure

6.4.1 Sample Preparation

1. Frozen sediment samples will be shipped from the logistics batching lab to the analysis lab.
2. Measure the bulk density of the sample.
3. Freeze dry or oven dry sediment samples.
4. Homogenize dried sediment for sample analysis.
5. Place dried sediment samples in plastic containers and store at room temperature until they are processed.

6.4.2 Method

1. Seal samples in ointment cans.
   a. Retrieve the dried and homogenized sample from the storage area.
   b. Select the appropriate size of ointment can for the amount of sample available. Use the largest size possible.
   c. Label the can with the laboratory sample number on both the top and bottom.
   d. Determine the tare weight of each can unit (top and bottom) using the analytical balance and record the weight on the worksheet.
   e. Place the can on a clean piece of paper (spilled sample can be recovered off of the paper if sample is limited). Transfer the sample into the ointment can in small increments. Tap the can gently to distribute the sample evenly in the can and to minimize air space in the sample. Repeat this process until the can is full but not over filled.
   f. Set the can lid on the bottom of the can. Do not push the bottom and top together at this point.
   g. Obtain the gross weight of the can with the sample in it and record the weight on the worksheet.
   h. Prepare the two-part epoxy as described on the package. Prepare no more than can be used in about 10 minutes.
   i. Carefully lift the cover off of the ointment can without disturbing the sample. Using the plastic stir stick that comes with the epoxy, coat the inside edge of the top of the can. Carefully replace the cover onto the bottom of the can. Continue to slide the top down onto the bottom of the can with a twisting motion until it is completely seated and the epoxy is spread over the entire edge.
   j. Apply more epoxy to the outside seam of the can. Orient the can so that the epoxy will sweep down into the joint between the top and bottom of the can. Set aside to dry.
   k. Inspect the epoxy seal after it has dried. Reapply more epoxy to any places along the can seam that look like they are not completely filled with epoxy.
   l. Record the date that the can was sealed on a worksheet and place the can in storage for a minimum of thirty days.

6.4.3 Calculation

1. See the instrument SOP

6.4.4 Calculating Efficiencies

1. Count the following standards to get 20,000 counts in the peak(s) of interest.
   a. Cs-137 S, M, L
   b. K-40 S, M, L
   c. Pb-210 S, M, L
   d. Ra-226 S, M, L
2. Run the supervisor program sed_eff.job on each of the resulting spectra. This job sets the ROIs for the spectrum.

3. Open the document “Det 4 sed eff.xls” on the gamma spectroscopy computer.
4. For each of the worksheets (small can, med can, large can).
5. At the end of the file put the current year in column A.
6. Copy and paste the 7 nuclide names, the associated yields and the formula from the “eff” column from in the previous year’s data.
7. For each spectrum:
   a. Open the spectrum.
   b. Select Edit | Sample Description to confirm the nuclide and geometry (S, M, L). Then click on Cancel.
   c. Select Setup | Full Energy Calibration.
   d. Select Edit Points.
   e. Click on Load ROIs.
   f. Click on Load Certificate File.
   g. Select the desired certificate file (k40_lg.ccf for the K-40 standard in the large can) and click on OK.
   h. Confirm the information from the certificate file, and modify if necessary. Click on OK.
   i. Click on OK-Fit. Depending on the Method selected there may be an error message that can be ignored.
   j. Transcribe the spectrum file name, end of count, energy, efficiency and error to the spreadsheet for each calibration point.
   k. Apply the formula from the “eff (cps/pCi)” column to the new data.
8. Open the Aptec Equation Editor.
9. For each geometry (S, M, L):
   a. Select File | Open Equation File and select the file: c:\windows\aptec\process\new_sed\sedcal4[sml].et
   b. Select “Save As” and save the current equation file as “eff4[sml]YY.et” where YY is the final 2-digits of the year that the efficiency was for.
   c. Edit the equation values 32-35 with the appropriate Ra226 eff(cps/pCi), Pb-210 eff, Cs-137 eff and K-40 eff respectively.

6.4.5 Analysis

- Determine the ^{210}Pb alpha activity using an argon-purged, low background, 2π proportional counter connected to a simple scaler.

6.5 Pertinent QA/QC Procedures

Reference counting standards are counted daily, and the counts plotted on control charts. Counts which fall outside the warning limits are evaluated for proper remedial action by lab personnel. Backgrounds are counted with each set of samples.

No external quality control samples are available.

6.5.1 Accuracy

At least one laboratory control spiked sample is analyzed with each sediment core. The lab control is made from a spiked salt sample. Spike the sample at least five times the method detection limit (2 to 5 pCi/g of Radium-226 and 5 to 25 pCi/g of lead 210). A modified Shewart accuracy chart is used to interpret these results.

6.5.2 Precision

The lab performs a replicate if sufficient sample quantity is available (if limited sediment is available, the lab will prioritize a replicate over a matrix spike).

If there is insufficient sample to perform a replicate analysis, note this on the calculation report.

6.5.3 Blank

At least one blank sample is analyzed with sediment core prepared from sodium chloride. An empty ointment may also be used.

6.5.4 Matrix

The lab performs a matrix spike if there is sufficient sample remaining after the initial analyses and the replicate is performed (see **Section 6.5.2**). If there is insufficient sample to perform a matrix spike analysis, note this on the calculation report.

Use the following criteria to determine whether or not the sample results may be reported:

- If all QC samples are within the control limits for that method, report all results.
- If any one of the QC results is out of the control limits, recount the suspect QC sample. If after recounting the QC sample, it is now within acceptable limits, report all results. If the QC sample remains out of control, the routine sample data may still be reported if their values are appropriate (refer to previous data for each collection site). The analyst must exercise careful judgment in this case. If there is any doubt about the results, reanalyze all samples.
- If more than one QC result is out of the control limits, repeat the analysis of the batch. If after repeating the batch of samples, the results of the QC samples are still not acceptable, the appropriate lab project manager will decide on the next course of action. The client report will list a qualifier for the out-of-control QC result(s) only if there is insufficient sample to repeat the analysis.
- If the blank sample is out of the control limits, investigate reagents and lab ware for the source of contamination. Sample results may be reported provided the above criteria for the precision, accuracy, and matrix spike have been met and that it obvious that there is no gross contamination.

The above criteria are based on the assumption that the instrumentation involved was properly calibrated and has met the QC requirements set in the instrument's SOP.

Acceptance Criteria for Efficiency Curves:

- First to Fifth order polynomial regressions are used for all curves. The regression co-efficient is calculated for each curve and must be 0.9 or better. Make a comparison to previous curves for the instrument in question to check for reasonableness.
- Data for all efficiency curves (except gamma spectroscopy) is imported into MicroCal Origin® Software. A plot is created for each curve which will display the regression co-efficient, date, isotope, and instrument.
- For gamma spectroscopy the data for the curve is statistically analyzed by the Canberra Software. Criteria for acceptance can be found in ESS RAD METHOD 006 (**APPENDIX E: SUPPORTING METHODS**)
- If the efficiency curve does not meet the above criteria as outlined in Appendix E, recount the curve. If the curve is still not acceptable, redo the curve. If the curve is still not acceptable, contact the vendor of the standard and if necessary purchase new standards. Consider contacting the instrument manufacturer for advice.

Point Calibrations (tritium, Ra-226, uranium, and Rn-222):

- Prepare a minimum of three points for the calibration. Average the values and make a comparison to previous values. Check the value by analyzing a laboratory control sample. The lab control can be in the next batch of actual samples, but it must meet the acceptance criteria for that method.
- If the lab control does not meet acceptance criteria, prepare at least three more calibrations, determine an average and analyze another lab control sample. If the results are still not satisfactory, contact the vendor of the standard and if necessary purchase new standards. Consider contacting the instrument manufacturer for advice.

6.6 Waste Disposal

Samples may only be discarded as specified in appropriate contracts or grants, see ESS RAD GENOP 011 SOP Sample Disposal (**APPENDIX E: SUPPORTING METHODS**). The samples must be neutralized before being discarded to the regular sewer.

Radioactive standards must be disposed of according to appropriate safety regulations. See ESS RAD GENOP 008 SOP Radioactive Standards (**APPENDIX E: SUPPORTING METHODS**).

7 SEDIMENT DIATOM METHODS

This method, adapted from protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water Quality Assessment (NAQWA) program (Charles et al. 2003), is used to process sediment core samples and identifies and enumerates diatoms. The method involves digestion of core samples, cover slip preparation, slide mounting, and microscopic examination of mounted diatoms.

Sediment diatom samples will be shipped on ice from field crews to a contract batching lab. The contract batching lab will freeze the samples send the batched samples to the analysis lab. Samples will arrive in the analysis lab frozen and can be held frozen for several months. Sediment diatom analysis labs 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

A qualified laboratory that has personnel with the appropriate training and experience in sediment diatom analysis may use this protocol to identify sediment diatoms. It is also important that all taxonomists maintain contact with other taxonomists through professional societies and other interactions, and keep abreast with the pertinent literature, since 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 enumeration and taxonomic precision, or consistency, as **percent difference in enumeration (PDE)** and **percent taxonomic disagreement (PTD)**, to 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.

7.2 Precautions

Wear appropriate clothing for safety precautions, such as safety glasses, nitrile gloves, rubber apron, long pants, etc. Use caution when working with acids. Always add acid very slowly and with great caution to avoid any unexpected reactions.

7.3 Equipment/Materials

Compound Microscope (with 10, 40, 100X objectives with 40-45X ocular, and epifluorescence capability)

Laboratory coat, gloves, and goggles

Fume hood

Micropipette (100µg and 1000 µg)

Pipette tips (100 µg and 1000 µg)

100-mL beaker

Hot plate

Nitric acid

DI water

Potassium dichromate

Fine-tipped vacuum hose

20-mL glass sample vials
18 x 18 mm cover slips
Compound microscope
Ceramic tiles
10% buffered formalin
Wax
Naphrax™
Wooden toothpicks
Forceps
Single-edge razor blade
Acetone
95% ethanol
Kimwipe® tissue
Microscope
pH test paper or Litmus paper
Sediment Diatom Sample Log-In Form (TBD)
Sediment Diatom Laboratory Sheet (TBD)
Labels (TBD)

7.4 Procedure

7.4.1 Sediment (Sediment Core Sample) Digestion

1. Transfer approximately 0.5 to 1.0 cc of either moist sediment to a 100-mL beaker. If dry, a small amount of water may be added to the sample (approximately 10 mL) to hasten disaggregation. The sample is to be analyzed quantitatively, which requires that the lab record the wet weight, volume, or dry weight of the sample to be processed.
2. Under a fume hood, set the hot plate dial to 200° C. Put on laboratory coat, gloves, and goggles. Place tray with all of the subsample beakers into the fume hood.
3. Nitric acid addition (hydrogen peroxide can be used in place of Nitric acid for this process) - Add enough nitric acid to each beaker to increase the volume to 50 mL. Initially, add acid very slowly and with great caution, anticipating that an unexpected reaction may take place. After determining that there is no possibility of a violent reaction, slowly and cautiously add the remainder of the acid to the samples. As a rule, the minimum sample to acid ratio should be 1:2.
4. Heat - Transfer the beakers to the hotplate and heat for 2 hours. Pay careful attention to samples while heating. If the volume drops too low, add DI water. When finished heating, add a dash of potassium dichromate to each beaker to catalyze the reaction. Keep adding small amounts until further reactions cease
5. Cool and dilute - After the beakers have cooled somewhat, transfer them back to the tray. Top off the beakers with DI water and allow diatom frustules to settle for 12 hours.
6. Siphon and add water - Using a fine-tipped vacuum hose, draw down the samples to approximately 50 mL. Siphon the water from the center of the water column under the surface. Make sure not to siphon the diatom layer off the bottom of the beaker. After siphoning, add DI water to replace the supernatant drawn off and wash the sides of the beaker to remove diatoms

adsorbed to the sides and top of the beaker. Let settle again for at least 9 hours. (As a rule, let settle 1 hour per 1 centimeter so the smallest diatoms can settle out).

7. Repeat siphoning - Repeat siphoning and addition step another 5 or 6 times and test the pH with pH test paper (or Litmus paper). When the samples are within the range of 6.5-7.5, the samples are ready for slide mounting.
8. Reduce volume - Draw the sample volumes down to between 25-50 mL, making sure not to remove diatoms.
9. Transfer and record volume - Transfer the remaining volume to labeled vials and record diatom volume after digestion on the laboratory processing sheet. Make sure to rinse all diatoms clinging to the beaker into the sample vial with DI water. If the full volume does not fit into the vial, allow the vial contents to settle for at least 12 hours and siphon off some of the supernatant. Transfer the remaining contents of the beaker into the vial, again making sure to rinse all remaining diatoms into the sample vial.

7.4.2 Preparing Cover Slips

1. Estimate volume to be placed on the cover slips - Starting with cleaned material contained within 20-mL glass vials, estimate the volume of suspended material that will need to be deposited ("dripped") on a cover slip to produce a slide of the appropriate cell density. Generally, between 5 and 10 diatom specimens should be present in a single high power microscope field (1000X).
2. To make the estimate, shake the cleaned material to ensure a homogeneous dispersion of cells within the 20-mL vial. Immediately open the vial and withdraw either a 25- or 50-µL sub-sample using the 0- to 100 µL adjustable pipettor. Place the subsample on a slide and cover it with an 18 x 18 mm cover slip.
3. Observe this preparation under a compound microscope at 50X magnification. Look at a number of fields and observe the density of cells.
4. Calculate the amount of material that would need to be dripped so that the density of cells seen at this magnification would be approximately 30 to 40 per field.
5. Sparse diatom samples - If a satisfactory slide could be made by increasing the concentration of cleaned diatom material by two to five times, then do this:
    a. Use a micropipette to remove the required amount of water from the vial of material after it has been allowed to settle for at least 8 hours.
    b. Record the concentration factor. If a concentration of cleaned material greater than five times is required, then re-subsample the original sample. Take a subsample of a size sufficient to prepare satisfactory slides. Use all remaining sample only if absolutely necessary.
    c. Digest the subsample and prepare a new vial of cleaned material. Repeat procedure above. If the concentration of cleaned material is still not sufficient, concentrate it, as described above (step 5). If still too dilute, combine the two vials of cleaned subsample materials. Record steps and volumes, and final concentration factor.
    d. If, after following the steps above to concentrate the cleaned material, the density of diatoms on a cover slip still does not meet the criteria of 30 to 40 cells per field at 400 – 450X magnification, then proceed to make the densest slide possible. As a general guideline, if accurate identifications are possible, and at least 100 specimens can be counted within four hours, the slide should be analyzed; otherwise do not analyze it.
6. Deposit cleaned material on cover slips - Use forceps to remove individual 18 x 18-mm cover slips. Place each cover slip on a marked space of the ceramic tile. Be sure the ceramic tile is clean and dry to avoid cross-contamination.

7. If the intended drip count is less than 600 µL, drip a small amount of distilled water onto the cover slip with a disposable pipette, sufficient to form a thin layer of water over the entire cover slip.
    a. Agitate the sample vial to a uniform dispersion and use the adjustable pipette to quickly withdraw the required amount from near the central portion of the sample. Dispense this material smoothly and carefully onto the layer of distilled water already on the slip. By alternately drawing material up into the pipette and dispensing it, you can achieve a homogeneous suspension on the cover slip.
    b. Take care to ensure that the suspension covers the entire surface of the cover slip, including the extreme edges of the corners. Should the cover slip overflow, discard the cover slip, and repeat the procedure with a freshly cleaned cover slip.
8. In the case where more than ~600 µL of original sample is required, the addition of distilled water is not necessary
    a. Dispense the sample directly onto the cover slip and mix.
    b. Take care to ensure that the suspension covers the entire surface of the cover slip, including the extreme edges of the corners. Should the cover slip overflow, discard the cover slip, and repeat the procedure with a freshly cleaned cover slip.
9. Drying samples - Discard the pipette tip when finished with each sample and make additional cover slips following the same procedure as in steps 5-7. Once the ceramic tile is loaded with prepared cover slips, the tile should remain undisturbed until the cover slips are dry.
    a. At this point, drying of the slips can proceed at room temperature (a period of several hours will be required), or gentle heat (warm to the touch only) may be applied to hasten evaporation (a crook-neck lamp with incandescent light bulb placed 15 - 30 cm over the drying plate is one option).
    b. Once completely dry, put the aluminum plate with cover slips on the hot plate that has been preheated to 250 to 300°F. Leave for 3 to 5 minutes. This procedure ensures that nearly all water is driven from the material on the cover slips and helps assure that the diatom frustules will adhere to the surface of the glass.
10. Check the slides - Remove the ceramic tile from the hotplate and inspect the cover slips. If the pattern of diatoms distributed on any of the cover slips is not even and smooth, they should be re-dripped.
11. Store samples - After a diatom slide is made and no additional sample is needed from the diatom vial, add 2-4 drops of 10% buffered formalin to each vial while working under a fume hood. Tightly cap the vials and seal them by immersing the top third of the vial in melted wax.

7.4.3 Mount Cover Slip on Microscope Slide

1. In a positive-draw fume hood, transfer a small amount of Naphrax™ (volume equivalent to ~2 to 4 drops of water) to the central portion of the etched side of the microscope slide using a rounded wooden splint or disposable pipette. Using a rounded wooden toothpick, distribute the Naphrax™ over an area approximately equivalent to the size of the cover slip.
2. Remove the appropriate cover slip from the aluminum plate with forceps, being careful to handle the cover slip only at the extreme corners. Invert the slip and place it gently on the Naphrax™ covered portion of the slide.
3. In a positive-draw fume hood, place the slide (cover slip up) on the hotplate and apply gentle heat until the evolution of bubbles resulting from the evaporation of the toluene solvent first occurs and then significantly diminishes.
4. Remove the slide from the hot plate, and, using the rounded toothpicks, gently position the cover slip and press it to form a thin, uniform layer of Naphrax™ beneath the entire cover slip.

Make sure that the edges of the cover slip are brought parallel to the edges of the microscope slide. Care must be taken at this stage not to press so hard as to damage or dislodge the diatoms or cause warping of the cover slip.

5. Set aside the mount to let the Naphrax™ become hard; use a single-edge razor blade to carefully trim any excess Naphrax™ which has been squeezed out from beneath the cover slip. Great care must be taken to avoid "lifting" the cover slip by inadvertently allowing the edge of the blade to move between the cover slip and the microscope slide.
6. Inside the fume hood, place the mount in successive baths of acetone and then ethanol for no more than 10 or 15 seconds each. Wipe the mount clean with a Kimwipe® tissue. Add a completed paper label to each slide before they are analyzed.

7.4.4 Identify and Enumerate 500 Diatom Valves

1. Variety-level resolution will be the taxonomic requirement for sediment diatoms. Labs should utilize the NARS taxonomic lists as your starting point, which are posted to the NARS Sharefile.
2. Scan slides at low to medium magnification (100X to 450X) to confirm that diatoms are evenly distributed on the cover slip, and are at a density appropriate for efficient counting. At high magnification (1000x), there should be 5-10 diatoms per field.
    a. If there are problems with dispersion or density that would compromise the quality and accuracy of the analysis, discuss these and have new slides made.
    b. Avoid counting diatoms in any disrupted areas of the mount, particularly edges that have optical aberrations. Always save any count data generated for a sample, even if the number of valves or frustules is low (e.g., <100).
3. Because slides may need to be recounted for QC purposes, it is very important to clearly demarcate the areas of a slide scanned during a count. After the preliminary slide examination, secure the slide in the mechanical stage and use the microscope's diamond scribe to etch a horizontal or vertical line on the cover slip to mark the edge of the first row to be counted.
    a. Rows are narrow rectangular areas (strips) of the slide adjacent to the scribed line, with width equal to the field of view. Start rows far enough from the cover slip edge to avoid optical distortion, and end them near the opposite cover slip edge where diatoms are no longer clearly visible.
    b. Locate a starting point near one end of the etched line and make a circle with the scribe. This denotes the starting point of the count. During the count, etch a circle around the last field counted in the first row and at the beginning and end of all other rows. Always check to make sure that etching is clearly visible so that circles and lines can be located easily by others.
4. When the line and first field are etched on the cover slip, and the first field is focused under oil immersion, begin to count 500 valves. Count all partial valves that are more than 50% of the valve or that contain unique features such as recognizable central areas or distinct ends. Count all valves and fragments that extend at least halfway into the field of view.
5. Stop counting when 600 valve count is reached. Clean the slide and store it properly.

7.5 Pertinent QA/QC Procedures

7.5.1 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 ≥ 90% identification as determined by QC checks, previous samples will be re-counted and identified.

7.5.2 External Taxonomic QC

1. On receipt of the data, the Indicator QC Coordinator randomly selects 10% of the samples for QC procedures. The lab will send out the original, etched slide with map for re-identification for each sample selected for QC. This will be sent to an external QC taxonomist (another experienced taxonomist who did not participate in the original identifications for those slides). The lab completes a chain-of-custody form and sends it with the sample. This will be considered a round-robin QC procedure.
2. The QC taxonomist performs the counting and identification procedure as described above, completing another copy of the Sediment Diatom Taxonomic Bench Sheet for each sample. Label each bench sheet with the term "QC Re-ID." As each bench sheet is completed, send it (email or fax) to the Indicator QC Coordinator.
3. 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 7.1 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 7.2 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 individuals in the larger of the two counts. The lower the PTD, the more similar the taxonomic results and the greater the overall taxonomic precision.
4. The recommendation for PDE is 15% or less.
5. 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 are investigated and logged for indication of error patterns or trends.
6. 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.

7.5.3 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 Sharefile.

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

Table 7.1 Laboratory quality control: sediment diatom indicator.

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
IDENTIFICATION			
Independent identification by outside taxonomist	All uncertain taxa	Uncertain identifications to be confirmed by expert in particular taxa	Record both tentative and independent IDs
Use standard taxonomic references	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
Prepare reference collection	Each new taxon per laboratory	Complete reference collection to be maintained by each individual laboratory	Lab Manager periodically reviews data and reference collection to ensure reference collection is complete and identifications are accurate
Round Robin External QC	10% of all samples completed per laboratory	PDE ≤ 15% PTD ≥ 85%	If PDE > 15%, implement recommended corrective actions. If PTD < 85%, implement recommended corrective actions.
DATA VALIDATION			
Taxonomic "reasonable-ness" checks	All data sheets	Genera known to occur in given lake or geographic area	Second or third identification by expert in that taxon

8 TRIAZINE PESTICIDE SCREEN

This method describes the application of enzyme linked immunosorbent assay (ELISA) to the determination of atrazine and related triazine occurrence and concentration in surface water samples. We use the Abraxis magnetic particle atrazine kit 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 triazine-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 triazines 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 0.03µg/L and the reporting limit is 0.05µg/L.

Cold triazine pesticide screen samples will be shipped on ice from the field crews to the contract batching lab. The contract batching lab will store samples in the refrigerator and send the batched samples to the analysis lab in coolers on ice. Samples will arrive in the analysis lab chilled and they can be held in a refrigerator or cold room for several weeks. Triazine pesticide screen analysis labs 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 based on the ELISA kit instructions.

8.1 Responsibility and Personnel Qualifications

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

8.2 Precautions

The stopping solution contains diluted sulfuric acid. 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.

8.2.1 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.

8.3 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

8.4 Procedure

8.4.1 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 if 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.

8.4.2 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.

8.4.3 Assay procedure

1. Label test tubes for standards, controls, and samples (**Table 8.1**).
2. Add 200 or 250 µL of the appropriate standard, control, or sample to the test tube.
3. Add 250 µL of Atrazine Enzyme Conjugate to each tube.
4. Mix the Atrazine Antibody Coupled Paramagnetic Particles thoroughly and add 500 µL 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 an additional time.
12. Remove the rack from the separator and add 500 µL 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 µL 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.

8.4.4 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 8.1 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

8.5 Pertinent QA/QC Procedures

8.5.1 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.

8.5.2 External QC

3. Analyze 10 provided spiked samples (blind sample) provided by the EPA HQ NARS QA Lead. After processing the samples, the laboratory will send the results to the EPA HQ NARS QA Lead. The results will be compared to the known concentrations and a determination made.

9 WATER CHEMISTRY and CHLOROPHYLL A

9.1 Analytical Parameters

A total of 18 parameters are determined from each bulk water chemistry sample collected (**Table 9.1**). 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 9.1 Water chemistry parameters measured for the 2012 National Lakes Assessment.

Analyte	Units	Comments
Conductivity	µS/cm at 25˚C	
Turbidity	NTU	
Acid Neutralizing Capacity (ANC)	µeq/L (20 µeq/L=1 mg as $CaCO_3$)	
Dissolved Organic Carbon (DOC)	mg C/L	
Ammonia (NH_3)	mg N/L	
Nitrate-Nitrate (NO_3-NO_2)	mg N/L	
Total Nitrogen (TN)	mg/L	
Total Phosphorus (TP)	µg P/L	
Sulfate (SO_4)	mg SO_4/L	
Chloride (Cl)	mg Cl/L	
Nitrate (NO_3)	mg N/L	May be obtained as part of nitrate-nitrite determination, or as a direct measurement (e.g., ion chromatography)
Calcium (Ca)	mg Ca/L	
Magnesium (Mg)	mg Mg/L	
Sodium (Na)	mg Na/L	
Potassium (K)	mg K/L	
Silica (SiO_2)	mg SiO_2/L	
Total Suspended Solids (TSS)	mg/L	
True Color	PCU	Performance objectives based on use of visual estimation method
Chlorophyll-*a*	µg/L (in extract)	

9.2 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), labs must notify the NARS IM Center (see **APPENDIX A: CONTACT INFORMATION**).

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 lab in NARS IM 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. Labs 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.

9.2.1 Water Chemistry Samples

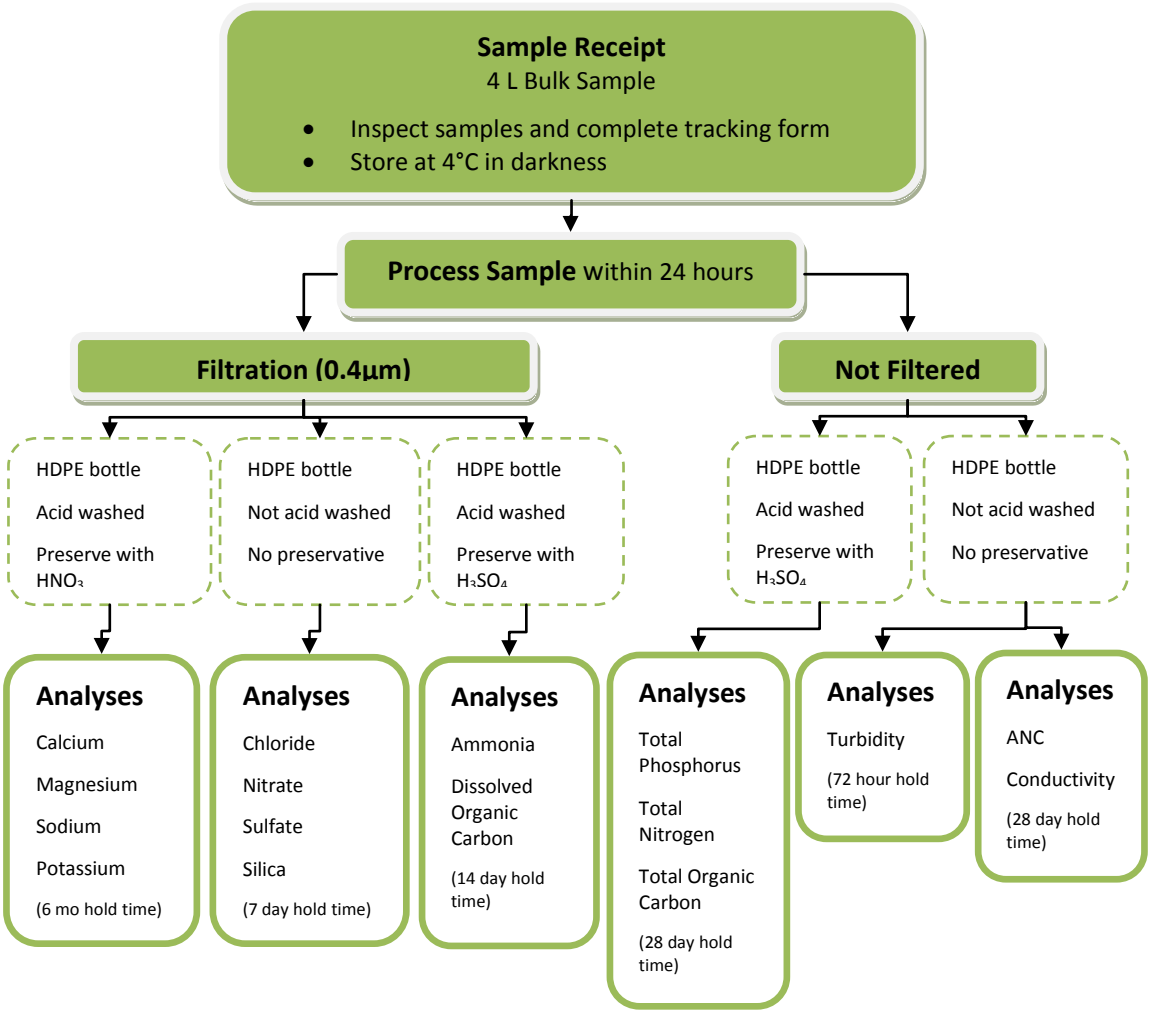

Figure 9.1 Water chemistry sample processing procedures

Figure 9.1 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 de-ionized (DI) water (ASTM Type II reagent water) five times before each use and in between samples. After placing a filter in the funnel unit, run approximately 100 mL of RO or DI water through the filter, with vacuum pressure, to rinse the filter. Discard the rinse water.

3. Place the appropriate sample bottle under the funnel unit and filter sample directly into the bottle. If a new filter is needed, remove the sample bottle, and rinse the new filter with 100 mL of RO or DI water before continuing.
4. After all filtered and unfiltered aliquots are collected, add ultra-pure acid (HNO_3 or H_2SO_4, depending on the analyte, see **Table 9.2**) 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 HNO_3) in a refrigerator at 4°C.

Table 9.2 Acid preservatives added for various analytes.

Preservatives	
H_2SO_4	**HNO_3**
DOC	Ca
NH_3	Mg
Total N	Na
Total P	K
NO_2-NO_3	

9.2.2 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 ± 2°C for no more than thirty days before analysis.

9.3 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 9.4**). 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 (≤ transition value) to relative (> transition value). For pH, the objectives are established for samples with lower H^+ (or OH^-) concentrations (pH between 5.75 and 8.25) and higher H^+ (or 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 (EPA ORD-Corvallis) are summarized in **Table 9.3**. Participating laboratories may use alternative analytical methods for each target analyte as long as they

can satisfactorily demonstrate the alternative method is able to achieve the performance requirements as listed in **Table 9.4**. Information is provided by the lab to the NLA Quality Team. The team reviews the information to determine whether the labs meet the necessary requirements. The information from this process is maintained in the NLA 2012 QA files by the EPA HQ NARS QA Lead.

Table 9.3 Summary of Analytical Methods Used by NLA 2012 (Central Laboratory, EPA ORD-Corvallis)

Analyte	Summary of Method[b]	References[c]	WRS SOP[d]
pH (lab)	Automated, using ManSci PC-Titrate w/ Titra-Sip autotitrator and Ross combination pH electrode. Initial pH determination for ANC titration	EPA 150.6 (modified)	WRS 16A.0 (April 2011)
Specific conductance @ 25°C	Electrolytic, Man-Tech TitraSip automated analysis OR manual analysis, electrolytic	EPA 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. EPA (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, EPA 180.1 U.S. EPA (1987)	WRS 16A.0 (April 2011) WRS 13A.3 (April 2011)
Total suspended solids (TSS)	Gravimetric, dried at 104 °C	EPA 160.2; APHA 209-C	WRS 14B.4 (February 2011)
True color (Hach Kit)	Visual comparison to calibrated glass color disk.	APHA 204 A (modified), EPA 110.2 (modified), U.S. EPA (1987)	WRS 15A.3 (April 2011)
Dissolved Organic Carbon (DOC)[e]	UV promoted persulfate oxidation to CO_2 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)	EPA 300.6; SW-846 9056A; APHA 4110B EPA 353.2 APHA 4500-NO_3-N-E Lachat 10-107-04-1-C	WRS 36A.0 (April 2011 WRS 40A.5 (May 2011)
Ammonia, as N (fresh waters)	FIA automated colorimetric (salicylate, dichloroisocyanurate)	Lachat 10-107-06-3-D	WRS 30A.4 (April 2011)
Silica, dissolved (SiO_2) 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)

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

[c] 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.

[d] WRS= Willamette Research Station. References are to laboratory SOP being used at central laboratory. Available upon request. (contact the Project Lead)

[e] 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).

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	EPA 300.6; SW-846 9056A; APHA 4110B	WRS 40A.5 (May 2011)
Major cations, dissolved calcium, sodium, potassium, magnesium	Inductively-coupled Plasma Atomic Emission Spectroscopy (ICP-AES) OR Flame AAS	EPA 200.7; EPA 6010B U.S. EPA (1987), EPA 215.1 EPA 273.1, EPA 258.1 EPA 242.1	WRS SOP 3.04 v3 (October 2011) WRS 50A.4 (March 2007)
Chlorophyll-a (Chl-a)	Extraction 90% acetone analysis by fluorometry	EPA 445.0 , EPA 446.0	WRS 71A.3 (April 2011)

9.4 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 labs.

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 2012 QAPP will be followed to ensure these LRLs are met for the NLA 2012.

9.4.1 Laboratory Performance Requirements

Table 9.4 summarizes the pertinent laboratory performance requirements for the water chemistry and chlorophyll A indicators.

9.4.2 Laboratory Quality Control Samples

Table 9.5 summarizes the pertinent laboratory quality control samples for the water chemistry and chlorophyll A indicators.

Table 9.4 Laboratory method performance requirements for water chemistry and chlorophyll-a sample analysis.

Analyte	Units	Potential Range of Samples[f]	Lower Reporting Limit[g]	Transition Value[h]	Precision Objective[i]	Bias Objective[j]
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 C/L	0.1 to 109	0.20	≤ 1 > 1	± 0.10 or ±10%	± 0.10 or ±10%
Ammonia (NH_3)	mg N/L	0 to 17	0.02 (1.4 µeq/L)	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Nitrate-Nitrate (NO_3-NO_2)	mg N/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 P/L	0 to 22,000	4	20	± 2 or ±10%	± 2 or ±10%
Sulfate (SO_4)	mg SO_4/L	0 to 5,000	0.50 (10 µeq/L)	2.5	± 0.25 or ±10%	± 0.25 or ±10%

[f] 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.

[g] The minimum 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.*

[h] 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*. 2nd ed. Royal Society of Chemistry, London, England.

[i] 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.

[j] 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.

Chloride (Cl)	mg Cl/L	0 to 5,000	0.20 (6 µeq/L)	1	± 0.10 or ±10%	± 0.10 or ±10%
Nitrate (NO_3)	mg N/L	0 to 360	0.02 (4 µeq/L)	0.1	± 0.01 or ±10%	± 0.01 ±10%
Calcium (Ca)	mg Ca/L	0.04 to 5,000	0.10 (5 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Magnesium (Mg)	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 Na/L	0.08 to 3,500	0.10 (4 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Potassium (K)	mg K/L	0.01 to 120	0.10 (2 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Silica (SiO_2)	mg SiO_2/L	0.01 to 100	0.10	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Total Suspended Solids (TSS)	mg/L	0 to 27,000	2	10	± 1 or ±10%	± 1 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 9.5 Laboratory quality control samples: water chemistry indicator.

QC Sample Type and Description	Analytes	Description	Frequency	Acceptance Criteria	Corrective Action
Laboratory/ Reagent Blank	All except TSS (For TSS, the lab will filter a known volume of reagent water and process the filters per method)		Once per day prior to sample analysis	Control limits ≤ 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.
Filtration Blank	All dissolved analytes	ASTM Type II reagent water processed through filtration unit	Prepare once per week and archive Prepare filter blank for each box of 100 filters, and examine the results before any other filters are used from that box.	Measured concentrations <LDL	Measure archived samples if review of other laboratory blank information suggest source of contamination is sample processing.
LT-MDL Limit Quality Control Check Sample (QCCS)	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.
Calibration QCCS	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	±10% or method criteria	Repeat QCCS analysis. Recalibrate and analyze QCCS. Reanalyze all routine samples (including PE and field replicate samples) analyzed since the last acceptable QCCS measurement.
Laboratory Duplicate	All analyses		One per batch	Control limits < precision	If results are below LRL: Prepare and analyze split

Sample				objective	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.
Standard Reference Material (SRM)	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.
Matrix Spike Samples	Only prepared when samples with potential for matrix interferences are encountered		One per batch	Control limits for recovery cannot exceed 100±20%	Select two additional samples and prepare fortified subsamples. Reanalyze all suspected samples in batch by the method of standard additions. Prepare three subsamples (unfortified, fortified with solution approximately equal to the endogenous concentration, and fortified with solution approximately twice the endogenous concentration).

9.4.3 Data Reporting, Review, and Management

Checks made of the data in the process of review and verification are summarized in **Table 9.6**. Data reporting units and significant figures are given in **Table 9.7**. The NLA 2012 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 9.6 Data validation quality control for water chemistry indicator.

Activity or Procedure	Requirements and Corrective Action
Range checks, summary statistics, and/or exploratory data analysis (e.g., box and whisker plots)	Correct reporting errors or qualify as suspect or invalid.
Review holding times	Qualify value for additional review
Ion balance: Calculate percent ion balance difference (%IBD) using data from cations, anions, pH, and ANC.	• If total ionic strength ≤100 µeq/L – %IBD ≤ ±25%. • If total ionic strength > 100 µeq/L – %IBD ≤±10%. • Determine which analytes, if any, are the largest contributors to the ion imbalance. Review suspect analytes for analytical error and reanalyze. – Flag = unacceptable %IBD • 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
Conductivity check: Compare measured conductivity of each sample to a calculated conductivity based on the equivalent conductance of major ions in solution (Hillman et al., 1987).	• If measured conductivity ≤ 25 µS/cm, – ([measured – calculated] ÷ measured) ≤ ±25%. • If measured conductivity > 25 µS/cm, – ([measured – calculated] ÷ measured) ≤ ±15%. • Determine which analytes, if any, are the largest contributors to the difference between calculated and measured conductivity. • Review suspect analytes for analytical error and reanalyze. • If analytical error is not indicated, qualify sample to attribute conductivity difference to unmeasured ions. Reanalysis is not required.
Review data 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 9.7 Data reporting criteria: water chemistry indicator.

Measurement	Units	No. Significant Figures	Maximum No. Decimal Places
DO	mg/L	2	1
Temperature	°C	2	1
pH	pH units	3	2
Carbon, total & dissolved organic	mg/L	3	1
ANC	µeq/L	3	1

Conductivity	µS/cm at 25 °C	3	1
Calcium, magnesium, sodium, potassium, ammonium, chloride, nitrate, and sulfate	µeq/L	3	1
Silica	mg/L	3	2
Total phosphorus	µg/L	3	0
Total nitrogen	mg/L	3	2
Nitrate-Nitrite	mg/L	3	2
Ammonia	mg/L	3	2
Turbidity	NTU	3	0
True color	PCU	2	0
TSS	mg/L	3	1
Chlorophyll a	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 9.1 Percent ion difference (%IBD)

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

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^+ 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 9.8**. For the conductivity check, equivalent conductivities for major ions are presented in **Table 9.9**.

Table 9.8 Constants for converting major ion concentration from mg/L to µeq/L

Analyte	Conversion from mg/L to µeq/L[k]
Calcium	49.9
Magnesium	82.3
Potassium	25.6
Sodium	43.5
Ammonia	55.4
Chloride	28.2
Nitrate	16.1
Sulfate	20.8

[k] Measured values are multiplied by the conversion factor.

Table 9.9 Factors to calculate equivalent conductivities of major ions.[l]

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	3.5×10^{5} [m]
Sodium	2.13	Hydroxide	1.92×10^{5} [b]
Ammonia	4.13	Bicarbonate	0.715
Chloride	2.14	Carbonate	2.82

[l] From Hillman et al. (1987).

[m] Specific conductance per mole/L, rather than per mg/L.

10 ZOOPLANKTON METHODS

This method is used to identify and enumerate species of lake zooplankton collected with vertical plankton net tows using both the NLA 2012 method and the NLA 2007 method. Macrozooplankton are counted from a sample using a 150 µm and 243 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 and 80 µm mesh nets.

Zooplankton samples will be preserved in the field with EtOH and shipped from field crews to a contract batching lab. The contract batching lab will send the batched samples to the analysis lab. Preserved samples can be held for several months, but zooplankton analysis labs 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.

10.1 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.

10.2 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.

10.3 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-µm 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-µm 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-µm 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% Ethanol
- 5% Sodium hypochlorite solution (unscented bleach)
- Rose Bengal stain dissolved in ethanol
- Dilute solution of laboratory detergent

10.4 Procedure

10.4.1 Zooplankton Stratified Splitting

1. Record all zooplankton samples received at the lab in a log book or sample log form (**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-µm or 80-µm mesh net through an 45-µm mesh sieve with de-ionized (DI) water to remove the EtOH; the second sample bottle, taken from the 150-µm or 243-µm mesh net, is rinsed through a 145-µm mesh sieve with de-ionized (DI) water to remove the EtOH. The two mesh size samples are treated as individual samples for processing and identification and recorded in the lab bench sheet with the sample number and corresponding mesh size.
2. Be sure to rinse the corresponding sample bottles thoroughly with reverse osmosis (RO)/DI/distilled water into the 45-µm mesh and 145-µm 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 see Zooplankton Enumeration Data Sheet).

10.4.2 Taxonomy Procedures

10.4.2.1 Taxonomic Level of Effort

Identify zooplankton to species where possible using 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).

10.4.2.2 Macrozooplankton Identification and Enumeration (Excluding Rotifers and Nauplii)

Macrozooplankton are counted and identified from samples collected with the coarse mesh (150 µm and the 243 µm) plankton net.

1. Species-level resolution will be the taxonomic requirement for macrozooplankton.
2. 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 Zooplankton Enumeration Data Sheet).
3. Concentrate the sub-sample by using the small sieve or the condensing tube and place in a circular (or other suitable) counting chamber.
4. Identify all macrozooplankton under a dissecting microscope and enumerate using a mechanical or electronic tally counter.
5. Count the first two sub-samples which likely contain 400 organisms (**Section 10.4.1**, 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 dominate 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.*

10.4.2.2.1 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% ethanol. 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.

10.4.2.2.2 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 appropriate column on spreadsheet, and put "na" (not applicable) in abundance column.

10.4.2.3 Microzooplankton (Rotifers, Nauplii, and Crustaceans)

Microzooplankton are counted and identified from samples collected with the fine mesh (50 µm and 80 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 10.4.1.5). 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.

10.4.2.3.1 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 (Appendix A-2). Make measurements on selected individuals at this time, and follow dominate, subdominant, and rare (Section 10.6). See measurement parameters for macro- and microzooplankton in sections 10.6.1 and 10.6.2, respectively.
3. After the counts are completed, measure the volume of the split used, including the volume of the aliquots, and record this information.

10.4.2.4 Measurement of Macrozooplankton and Microzooplankton

10.4.2.4.1 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.*

10.4.2.4.2 Rotifers

Measure at least 20 encounters per species as follows:

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

10.5 Calculating and Reporting

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

10.5.1 Volume of water filtered

Equation 10.1 Volume of water filtered.

$$V = L \times A$$

where:

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

10.5.2 Macrozooplankton Densities

Equation 10.2 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)

10.5.3 Microzooplankton Densities

Equation 10.3 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_a = Number of 1mL aliquots examined
V_s = Volume of sub-samples from which aliquots were taken
S = Spilt factor
V = Volume of water filtered (from above calculation)

10.5.4 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).

10.5.5 Results of Laboratory Processing, Sample Archiving

Prepare a completed data sheet (Attachment or Table) 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). Pertinent QA/QC Procedures

10.6 Pertinent QA/QC Procedures

10.6.1 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 lab. 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 10.4 Relative percent difference (RPD).

$$RPD = \frac{|n_1 - n_2|}{(n2 + n_2)/2} \times 100$$

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 10.5 Root mean square error (RMSE) or standard error of estimate.

$$RMSE = \sqrt{\frac{\sum_{j-1}^{k}\sum_{i-1}^{n_1}\left(y_{ij} - \bar{y}_j\right)^2}{\sum df_{1\ldots k}}}$$

where y_{ij} is the i^{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).

10.6.2 Taxonomic QC

10.6.2.1 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 ≥ 90% identification as determined by QC checks, previous samples will be re-counted and identified.

10.6.2.2 External Taxonomic QC

1. On receipt of the data after initial identification, approximately 10% of the samples (for each lab) 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 lab to a QC taxonomist for complete re-identification and re-enumeration. The lab will complete and send with the samples a chain-of-custody 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 10.6 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 ≤ 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. 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.

10.6.2.3 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.

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 Sharefile.

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

Table 10.1 Laboratory quality control: zooplankton indicator

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
IDENTIFICATION			
Independent identification by outside taxonomist	All uncertain taxa	Uncertain identifications to be confirmed by expert in particular taxa	Record both tentative and independent IDs
Use standard taxonomic references	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
Prepare reference collection	Each new taxon per laboratory	Complete reference collection to be maintained by each individual laboratory	Lab Manager periodically reviews data and reference collection to ensure reference collection is complete and identifications are accurate
External QC	10% of all samples completed per laboratory	Efficiency (PTD) ≥ 85%	If PTD < 85%, implement recommended corrective actions.
DATA VALIDATION			
Taxonomic "reasonable-ness" checks	All data sheets	Genera known to occur in given lake or geographic area	Second or third identification by expert in that taxon

11 RESEARCH INDICATOR: SEDIMENT MERCURY

Total Mercury (Hg) by Direct Combustion Analysis

All sample processing, analysis, and reporting will be conducted by at the U.S. Geological Survey's Mercury Research Laboratory (MRL) located in Middleton, Wisconsin. An abbreviate description of the total Hg analysis procedures used for this study is provided below, however, the details of all sample analysis procedures for the MRL can be found at http://wi.water.usgs.gov/mercury-lab. In brief, the USGS MRL will analyze these samples as described in the MRL Standard Operating procedures for total Hg in bed sediment - Analysis of Total Mercury in Solid Samples by Atomic Adsorption following Direct Combustion with the Nippon MA-2 Mercury Analyzer, which is principally the same as USEPA Method 7473.

Samples will be shipped overnight from field crews to WRS, where they will be frozen and batch shipped to MRL. Samples should arrive to the lab frozen. These samples will be stored at -15 C, where the temperature is monitored daily. If the temperature is not at the correct level, the samples will be qualified as suspect for all analyses. We do not know of holding time studies for frozen mercury samples; however frozen certified reference material (CRM) for Hg is available through the National Institute of Standards and Technology (NIST) and is stable for a duration of 9 years.

11.1 Principle of Operation

Solid sample is combusted at high temperature (850 °C) in the presence of interference-reducing reagents, releasing mercury from the matrix as reduced gaseous mercury. In the resulting gas, matrix interference is further eliminated by catalytic treatment, adjusted to appropriate pH in a phosphate buffer, and then passed through a gold amalgam trap to quantitatively capture gaseous mercury. Lastly, the gold trap is heated, releasing the bound mercury into the sample stream, and detected by cold vapor atomic adsorption.

11.2 Instrument Operation

This document is intended as an additional standard operating procedure (SOP) designed to guide the user through mercury analysis specific to the Wisconsin District Mercury Laboratory. A condensed version is also provided following the detailed SOP, and is intended as a quick reference bench guide for the analyst. However, the analyst is required to be familiar with the detailed SOP as well as the original user's manual provided by Nippon which will be referred to when appropriate.

11.2.1 Start up

If the instrument is off, turn it on with the switch near the mains. If necessary, start the software (click on shortcut located on the desktop, "MA2000") and open the appropriate (HIGH CAL or LOW CAL) template file. The template files include a standard curve that was successfully used on the instrument for the previous analysis; it is not necessary to calibrate the instrument with every use.

The LOW CAL file operates from 0.2 – 20 ng and is generally used for sediment analysis, while the HIGH CAL file operates from 2 – 200 ng and is generally used for biological analysis. Choose the correct analysis mode ("low mode" for LOW CAL function, and "high1 mode" for HIGH CAL function) by clicking the drop down menu "run", select "mode", and choose radio button. On the instrument diagram, make sure that the heat mode is in "mode 2" and the measurement mode is correct for the intended analysis. If the instrument is "cold" allow it to come up to operating temperature.

Empty the gas washing bottle (left bottle) of buffer solution, and drain residual moisture from the dehumidifying bottle (right bottle). Fill the gas washing bottle with 2 cm of buffer solution, being sure to leave the dehumidifying bottle open to vent head space (otherwise buffer solution will be forced upstream into the end cap and require shut down and cleaning). If necessary, remove combustion boats from sample tray, empty the spent reagent-sample mixture into a large Ziploc bag, and vacuum residual reagent dust from boats. Gently vacuum any reagent dust that has collected on interior components of the instrument, including the sample changing tray and surrounding areas (tray removal function possible in the "run" drop down menu). Clear the instrument of residual mercury by running the purge function (select the PURGE option in the sample table from the NAME drop down menu). Repeat purge until at baseline level (peak area < 0.005).

11.2.2 Preparation for Sample Analysis

It is important that the combustion boats are mercury and acid free. Prior to use, newly acid washed boats should be heated in the oven at 550 °C for 2 hours, and boats not used in the previous 3 days should be clean burned in the instrument. If the boats have been recently used, randomly select 10% of combustion boats (3-6) to be used for the analysis and clean burn (without reagents) them to ensure that there is no significant carryover (peak area < 0.01) from previous analyses. If the boats fail this criterion, repeat with 3-6 additional boats, and if contamination persists the entire lot of boats needs to be clean burned before use.

When the boats are clean analyze three reagent blanks, at least three relevant standard reference material (SRM) samples, and two check standards. Analysis requires the addition of solid reagents to the combustion boats and is further described in chapter 5 of the instruction manual. For the analysis of standards, add additive B, 10 – 1000 µl of standard, additive B to cover, and finally fill the boat with additive M. For the analysis of solid samples, add additive M, 10 – 50 mg sample, additive M covering the sample, additive B covering that, and finally fill the boat with additive M. Following analysis, if the initial reagent blanks are sufficiently low (< 0.05 ng/boat), the SRM is within the accepted range (± 20% recovery), and the check standard recovery is within 10%, proceed with sample analysis. In the case of an elevated reagent blank, and SRM or check standard recovery failure, repeat the measurement. A repeated failure rules out analyst error and indicates that the instrument is not performing properly; samples should not be analyzed until the issue is corrected.

11.2.3 Sample Analysis

Samples may be analyzed once the preceding instrumental control has been demonstrated. Analytical sample mass should be 10 – 50 mg. Every analytical batch of ten samples will include at least: one sample analyzed in triplicate, one SRM analysis, and two reagent blanks. The reagent blanks, preceded by an instrumental purge, are located in the middle and at the end of the sample set. If necessary, additional purges may be added to a batch.

Table 11.1 Performance requirements for total mercury and methyl mercury

Analyte	Units	Reporting limits	Precision Objective	Bias Objective
Total Mercury	ng/analytical aliquot	0.3	±10%	±10%
Methyl Mercury	ng/g	0.08	±10%	±10%

11.3 Pertinent QA/QC Procedures

11.3.1 Standard Reference Material

Recovery of the reference material must be within 80-120% of its certified value. Repeat the SRM in the case of failure. A second failure indicates the method is not performing properly and the problem needs to be corrected and the samples repeated.

11.3.2 Sample Precision

The relative standard deviation of samples in triplicate should be less than 15%. In the case of failure repeat the sample (if possible) in addition to another sample from the same set in triplicate. Repeated triplicate failure should be brought to the attention of the quality assurance officer.

11.3.3 Sample Carryover

The purge function of the instrument clears the sample train of residual mercury and indicates the level of carryover from previous sample analyses. A purge mass should not exceed 10% of the mass of mercury measured in any previous sample, up to the previous purge. When a purge exceeds 10% of a previous mercury mass, repeat that sample in a subsequent batch bracketed with purges. If significant carryover persists in a sample set, mercury concentrations tend to be extremely low, and/or sample volume is extremely limited, increase the frequency of purges to reduce inter-sample carryover.

11.3.4 Reagent Blank

Reagent blanks analyzed before and throughout analytical batches measure the mercury concentration present in the additives M and B. If any one of the three initial reagent blanks exceeds 0.05 ng/boat, reanalyze three reagent blanks using the same boats. Repeated failure of initial reagent blanks indicate the additive is contaminated and should be combusted again before future use. Reagent blanks throughout the analytical batch are preceded by an instrumental purge to clear the sample train of residual mercury, reducing sample carryover. Reagent blanks within an analytical batch exceeding 0.05 ng/boat indicate contamination of additive source or persistent systemic contamination. Repeat the preceding samples of a failed reagent blank up to the last passing reagent blank (< 0.05 ng/boat) or instrumental purge with a peak area < 0.005; if sample carryover is suspected in this batch, the samples should be bracketed with purges. If reagent blanks continue to fail the repeated analysis, the additive has become contaminated and should be combusted.

11.3.5 Instrument Calibration

A standard curve should be (1) created with mercury masses appropriate to the measurement mode, (2) calculated with a polynomial best fit equation with an intercept of zero, and (3) have an r2 value greater than 0.995. The mass of mercury in analyzed samples should occur within the levels of the standard curve. Instrumental response tends to be relatively stable over multiple days; therefore daily calibration is not necessary. However, instrumental calibration should be verified (± 10%) prior to sample analysis by analysis of a known mass of mercury from a standard solution.

11.3.6 Interferences

The instrument is extremely sensitive to acid and free halogens, which degrade the catalyst and gold trap. It is very important to reduce/eliminate exposure to these factors throughout analysis and storage. Saline sediments (such as marine sediments) and potentially acidified samples should be analyzed sparingly with the Nippon or with an alternative method.

11.3.7 Reagents

Before use, heat reagents to 750 C for 1 hr in 250 ml crucible to volatilize residual mercury and water. Leave in furnace until cool and transfer back into original container if not immediately used.

11.3.8 Standard Solution

Mercury standards are prepared in a 0.001% L-cysteine, 0.2% Nitric acid solution; do not use standards prepared in any other matrix as that acids and free halogens substantially interfere with instrument performance. Standards solutions of 10, 100, and 10000 ng/ml meet most analytical needs of the instrument.

11.3.9 Data Capture and Processing

Data from analysis appears in the run list in the sample page and is written to the "DEPOSIT.MA" file. In the run list, copy the columns for sample ID, sample mass, and mercury mass. Paste this data into the appropriate excel spread sheet template (HIGH CAL or LOW CAL) for processing and save with the file name as the analytical date (012309.xxx). Following analysis, save the DEPOSIT.MA file as the same name.

11.3.10 Maintenance Schedule

Daily gently vacuum the interior components of the instrument to minimize dust build up. Change buffer solution. Visually inspect the downstream components (end cap, bubblers, gold amalgam trap, cell, and connecting tubing) for deposits and clean or replace as necessary.

Monthly acid-wash the end cap and inspect the end of the combustion tube for deposited material. Replace combustion tube and clean as necessary. Acid-wash combustion boats as described below.

11.3.11 Acid Washing

All acid-washing is done in a 10% HNO3 solution. Wash glass equipment and ceramic combustion boats for at least 2 and 24 hours, respectively. Rinse glass equipment well with mercury-clean water and let dry before use. Following acid washing, boats need to be soaked in mercury-clean water for a minimum of 24 hours to become fully rinsed, dried for 3 days, and heated to 550 C for 2 hours before use.

12 RESEARCH INDICATOR: DISSOLVED CARBON

Dissolved Carbon laboratory procedures will not be included in this manual. We are working with colleagues at USGS in the Land Carbon Project to have these samples processed and analyzed.

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APPENDIX B: LABORATORY REMOTE EVALUATION FORMS

Document Request Form – **Chemistry** Labs

EPA 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 2012 National Lakes Assessment (NLA), the Quality Assurance Team has been requested to conduct a technical assessment to verify quality control practices in your laboratory and its ability to perform **chemistry** analyses under this project. Our review will be assessing your laboratory's ability to receive, store, prepare, analyze, and report sample data generated under EPA's 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 labs will need to complete the following form:

- ☐ A *signature* on the attached Lab Signature Form indicates that your lab will follow the quality assurance protocols required for chemistry labs conducting analyses for the 2012 NLA.

In order for us to determine your ability to participate as a lab 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 lab can provide either documentation of a prior audit or accreditation, no other documentation is needed. If ***neither*** of above is complete, please provide the following information.

- ☐ A copy of your Laboratory's *Quality Manual*
- ☐ *Standard Operating Procedures* (SOPs) for your lab for each analysis to be performed (if not covered in 2012 NLA Lab Manual)
- ☐ Other documentation supporting your lab's ability to meet the required level of data quality (if available)

This documentation may be submitted electronically via e-mail to pollard.amina@epa.gov. Questions concerning this request can be submitted to pollard.amina@epa.gov (202-566-2369) or johnson.marshal@epa.gov (202-564-2858).

Lab Signature Form – Chemistry Labs

I _____certify that the _____lab, 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 2012 NLA Lab Operations Manual (or equivalent). If using equivalent procedures, please provide procedures manual.
2.) Read and abide by the 2012 NLA 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 Lab 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, 2013 or as otherwise negotiated with EPA.
6.) Participate in a lab technical assessment or audit if requested by EPA NLA staff (this may be a conference call or on-site audit).

Signature _____ Date _____

Document Request Form - **Biology** Labs

EPA 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 2012 National Lakes Assessment (NLA), 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 EPA's 2012 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): reconciliation exercises and/or a site visit. All labs will need to complete the following form:

- ☐ A *signature* on the attached Lab Signature Form indicates that your lab will follow the quality assurance protocols required for chemistry labs conducting analyses for the 2012 NLA.

In order for us to determine your ability to participate as a lab 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 lab can provide either documentation of a prior audit or accreditation, no other documentation is needed. If ***neither*** of 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 lab for each analysis to be performed (if not covered in 2012 NLA Lab Manual)
- ☐ Other documentation supporting your lab's ability to meet the required level of data quality (if available)

This documentation may be submitted electronically via e-mail to pollard.amina@epa.gov. Questions concerning this request can be submitted to pollard.amina@epa.gov (202-566-2369) or johnson.marshal@epa.gov (202-564-2858).

Lab Signature Form – Biology Labs

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

7.) Utilize procedures identified in the 2012 NLA Lab Operations Manual (or equivalent). If using equivalent procedures, please provide procedures manual.
8.) Read and abide by the 2012 NLA Quality Assurance Project Plan (QAPP) and related Standard Operating Procedures (SOPs).
9.) Have an organized IT system in place for recording sample tracking and analysis data.
10.) Use taxonomic standards outlined in the 2012 NLA Lab Manual.
11.) Participate in taxonomic reconciliation exercises during the field and data analysis season, which include conference calls and other lab reviews.
12.) Provide data using the template provided in the Lab Operations Manual.
13.) 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, 2013 or as otherwise negotiated with EPA.
14.) Participate in a lab technical assessment or audit if requested by EPA NLA staff (this may be a conference call or on-site audit).

Signature _____ Date _____

APPENDIX C: 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				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				

Phytoplankton Measurement Data Sheet

Sample #_____ Lake_____ Lab #_____

Date Collected_____ Depth of tow_____ Analyzed by_____

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Zooplankton Sample Log In Form

Date Received	Sample Type	Sample Number	Lake Name	Station	Depth	Laboratory Tracking #	Notes

Zooplankton Enumeration Data Sheet

Sample # _____ Lake _____ Lab # _____

Date Collected _____ Depth of tow _____ Analyzed by _____

Working Volume (mL) _____ Milliliters in subsample (rotifers) _____ Split _____

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

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

Zooplankton Measurement Data Sheet

Sample #_____ Lake_____ Lab #_____

Date Collected_____ Depth of tow_____ Analyzed by_____

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

APPENDIX D: REPORTING TEMPLATES

Templates will be provided on the NARS Sharefile.

APPENDIX E: SUPPORTING METHODS

ESS RAD METHOD 006 Preparation of Spiked Samples for Efficiency Calibration

ESS RAD GENOP 011 SOP Sample Disposal

ESS RAD GENOP 008 SOP Radioactive Standards