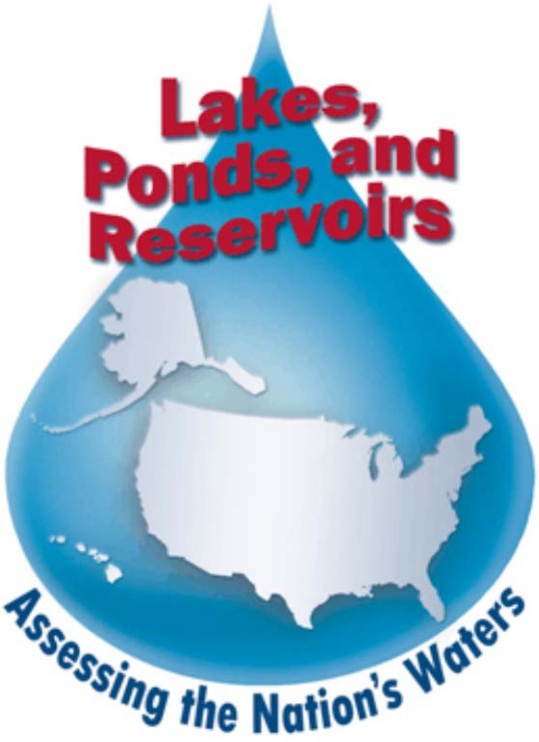
United States Environmental Protection Agency
Office of Water
Office of Environmental Information
Washington, DC
EPA 841-B-07-003

Survey of the Nation's Lakes

Quality Assurance Project Plan



October 2009

Survey of the Nation's Lakes (Lakes Survey)
Quality Assurance (QA) Project Plan

QUALITY ASSURANCE PROJECT PLAN
REVIEW & DISTRIBUTION ACKNOWLEDGMENT AND
COMMITMENT TO IMPLEMENT

for

Survey of the Nation's Lakes

We have read the QAPP and the methods manuals for the Lakes Survey listed below. Our agency/organization, agrees to abide by its requirements for work performed under the Lakes Survey (under CWA 106).

Quality Assurance Project Plan □
Field Operations Manual □
Site Evaluation Guidelines □
Laboratory Methods Manual □

Print Name _____

Title _____
(Cooperator's Principal Investigator)

Organization_____

_____ _____
Signature Date

NOTICE

The complete documentation of overall Lakes Survey project management, design, methods, and standards is contained in four companion documents, including:

- *Survey of the Nation's Lakes: Quality Assurance Project Plan (EPA 841-B-07-003)*
- *Survey of the Nation's Lakes: Lake Evaluation Guidelines (EPA 841-B-06-003)*
- *Survey of the Nation's Lakes: Field Operations Manual (EPA 841-B-07-004)*
- *Survey of the Nation's Lakes: Laboratory Methods Manual (EPA841-B-07-005)*

This document (Quality Assurance Project Plan) contains elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for the Lakes Survey. Methods described in this document are to be used specifically in work relating to the Lakes Survey. All Project Cooperators should follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. More details on specific methods for site evaluation, field sampling, and laboratory processing can be found in the appropriate companion document(s).

The suggested citation for this document is:

> USEPA. 2009 (Final). Survey of the Nation's Lakes: Integrated Quality Assurance Project Plan. EPA/841-B-07-003. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.

DISTRIBUTION LIST

This QA Project Plan and associated manuals or guidelines will be distributed to the following: EPA, States, Tribes, universities, and contractors participating in the Lakes Survey. EPA Regional Lake Survey Coordinators are responsible for distributing the Lakes Survey QA Project Plan to State and Tribal Water Quality Agency staff or other cooperators who will perform the field sampling and laboratory operations. The Tetra Tech and Great Lakes Environmental Center QA Officers will distribute the QA Project Plan and associated documents to participating project staff at their respective facilities and to the project contacts at participating laboratories, as they are determined.

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1.0 PROJECT PLANNING AND MANAGEMENT

1.1 Introduction

Several recent reports have identified the need for improved water quality monitoring and analysis at multiple scales. In 2000, the General Accounting Office (USGAO 2000) reported that EPA, states, and tribes collectively cannot make statistically valid inferences about water quality (via 305[b] reporting) and lack data to support key management decisions. In 2001, the National Research Council (NRC 2000) recommended EPA, states, and tribes promote a uniform, consistent approach to ambient monitoring and data collection to support core water quality programs. In 2002, the H. John Heinz III Center for Science, Economics, and the Environment (Heinz Center 2002) found there is inadequate data for national reporting on fresh water, coastal and ocean water quality indicators. The National Association of Public Administrators (NAPA 2002) stated that improved water quality monitoring is necessary to help states and tribes make more effective use of limited resources. EPA's Report on the Environment 2003 (USEPA 2003) says that there is not sufficient information to provide a national answer, with confidence and scientific credibility, to the question, 'What is the condition of U.S. waters and watersheds?'

In response to this need, the U.S. Environmental Protection Agency (EPA) Office of Water in partnership with states and tribes has begun a program to assess the condition of the nation's waters via a statistically valid approach. The current assessment, the Survey of the Nation's Lakes (referred to as Lakes Survey throughout this document), builds upon the Wadeable Streams Assessment implemented by EPA to monitor and assess the condition of the nation's wadeable stream resource. The Lakes Survey effort will provide important information to states and the public about the condition of the nation's lake resource and key stressors on a national and regional scale.

EPA developed this Quality Assurance Project Plan (QAPP) to support the states and tribes participating in this project. The plan contains elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for the Lakes Survey. EPA recognizes that states and tribes may have added elements, such as supplemental indicators, that are not covered in the scope of this integrated Quality Assurance Project Plan. EPA expects that any supplemental elements are addressed in a separate approved QAPP or an addendum to this QAPP by the states and tribes or their designee.

As a cooperative effort between states, tribes, and federal agencies, a broad-scale study to assess the condition of the Nation's lakes with both confidence and scientific credibility is made possible. Through this survey, states and tribes have the opportunity to collect data which can be used to supplement their existing monitoring programs or to begin development of new programs. The Lakes Survey has two main objectives:

- Estimate the current status, trends, and changes in selected trophic, ecological, and recreational indicators of the condition of the Nation's Lakes with known statistical confidence.

- Seek associations between selected indicators of natural and anthropogenic stresses and indicators of ecological condition.

1.2 Lakes Survey Project Organization

The responsibilities and accountability of the various principals and cooperators are described here and illustrated in Figure 1-1. The overall coordination of the project will be done by EPA's Office of Water (OW) in Washington, DC, with support from the Western Ecological Division (WED) of the Office of Research and Development (ORD) in Corvallis, Oregon. Each EPA Regional Office has identified a Regional EPA Coordinator who is part of the EPA team providing a critical link with state and tribal partners. Cooperators will work with their Regional EPA Coordinator to address any technical issues. A comprehensive quality assurance (QA) program has been established to ensure data integrity and provide support for the reliable interpretation of the findings from this project. Technical Experts Workgroups will be convened to decide on the best and most appropriate approaches for key technical issues, such as: (1) the selection and establishment of reference conditions based on least-disturbed sites and expert consensus for characterizing benchmarks for assessment of ecological condition; (2) selection and calibration of ecological endpoints and attributes of the biota and relationship to stressor indicators; (3) a data analysis plan for interpreting the data and addressing the objectives in a nationwide assessment; and (4) a framework for the reporting of the condition assessment and conveying the information on the ecological status of the Nation's lakes.

Contractor support is provided for all aspects of this project. Contractors will provide support ranging from implementing the survey, sampling and laboratory processing, data management, data analysis, and report writing. Cooperators will interact with their Regional EPA Coordinator and the EPA Project Leader regarding contractual services.

The primary responsibilities of the principals and cooperators are as follows:

EPA Project Leader- Carol Peterson

- Provides overall coordination of the project and makes decisions regarding the proper functioning of all aspects of the project.
- Makes assignments and delegates authority, as needed to other parts of the project organization.

Alternate EPA Project Leader- Steve Paulsen

- Assists EPA Project Leader with coordination and assumes responsibility for certain aspects of the project, as agreed upon with the EPA Project Leader.
- Serves as primary point-of-contact for project coordination in the absence or unavailability of EPA Project Leader.
- Serves on the Technical Experts Workgroup and interacts with Project Facilitator on technical, logistical, and organizational issues on a regular basis.

Regional EPA Coordinator

- Assists EPA Project Leader with regional coordination activities.
- Serves on the Technical Experts Workgroup and interacts with Project Facilitator on technical, logistical, and organizational issues on a regular basis.
- Serves as primary point-of-contact for the Cooperators.

Technical Experts Workgroup(s) - States, EPA, academics, other federal agencies

- Provides expert consultation on key technical issues as identified by the EPA Coordination team and works with Project Facilitator to resolve approaches and strategies to enable data analysis and interpretation to be scientifically valid.

Tetra Tech (Tt) Project Facilitator – Michael Barbour

- A contractor who functions to support implementation of the project based on technical guidance established by the EPA Project Leader and Alternate EPA Project Leader
- Primary responsibility is to ensure all aspects of the project, i.e., technical, logistical, organizational, are operating as smoothly as possible.
- Serves as point-of-contact for questions from field crews and cooperators for all activities.

Great Lakes Environmental Center (GLEC) Technical Representative- Dennis McCauley

- Provides contractor support to the project and works with Project Facilitator to ensure all needs for contractor support are covered.

Cooperator(s)

- Under the scope of their assistance agreements, plans and executes their individual studies as part of the cross jurisdictional Survey of the Nation's Lakes, and adheres to all QA requirements and standard operating procedures (SOPs).
- Interacts with the Grant Coordinator, Project Facilitator and EPA Project Leader regarding technical, logistical, organizational issues.

Field Sampling Crew Leader

- Functions as the senior member of each Cooperator's field sampling crew and the point of contact for the Field Logistics Coordinator.
- Responsible for overseeing all activities of the field sampling crew and ensuring that the Project field method protocols are followed during all sampling activities.

Field Logistics Coordinator

- A contractor who functions to support implementation of the project based on technical guidance established by the EPA Project Leader and Alternate EPA Project Leader serves as point-of-contact for questions from field crews and cooperators for all activities.
- Tracks progress of field sampling activities.

Information Management Coordinator

- A contractor who functions to support implementation of the project based on technical guidance established by the EPA Project Leader and Alternate EPA Project Leader oversees all sample shipments and receives data forms from the Cooperators.
- Oversees all aspects of data entry and data management for the project.

EPA QA Officer

- Functions as the primary officer overseeing all QA and quality control (QC) activities.
- Responsible for ensuring that the QA program is implemented thoroughly and adequately to document the performance of all activities.

QA Project Officer(s)

- Oversee(s) individual studies of cooperators (assistance recipients).
- Interacts with EPA Project Leader and Project Facilitator on issues related to sampling design, project plan, and schedules for conduct of activities.
- Collects copies of all official field forms, field evaluation checklists and reports.
- Oversees and maintains records on field evaluation visits, but is not a part of any one sampling team.

Tetra Tech (Tt) QA Officer

- The contractor QA Officer who will supervise the implementation of the QA program.
- Directs the field and laboratory audits and ensures the field and lab auditors are adequately trained to correct errors immediately to avoid erroneous data and the eventual discarding of information from the assessment.

Great Lakes Environmental Center (GLEC) QA Officer

- Provides support to the Tt QA Officer in carrying out the QC checks and documenting the quality of the activities and adherence to specified procedures.

EPA Headquarters Indicator Team

- Oversees the transfer of samples and related records for each indicator.
- Ensures the validity of data for each indicator.

1.2.1 Project Schedule

Training and field sampling will be conducted in 2007. Sample processing and data analysis will be completed by 2008 in order to publish a report the following year. Figure 1-2 gives an overview of the major tasks leading up to the final report.

1.3 Scope of QA Project Plan

This QA Project Plan addresses the data acquisition efforts of Lakes Survey, which focuses on the 2007 sampling of lakes across the United States. Data from approximately 1000 lakes (selected with a probability design) located within the contiguous 48 states will provide a comprehensive assessment of the Nation's lakes. Companion documents to this QAPP that are relevant to the overall project include *Survey of the Nation's Lakes: Site Evaluation Guidelines, Survey of the Nation's Lakes: Field Operations Manual*, and *Survey of the Nation's Lakes: Laboratory Methods Manual.*

1.3.1 Overview of Field Operations

Field data acquisition activities are implemented for the Lakes Survey, based on guidance developed by EMAP (Baker and Merritt 1990), through the direction of a steering committee comprised of various state, tribal, and regional agencies. Funding for states and tribes to conduct field data collection activities are provided by EPA under Section 106 of the Clean Water Act. Survey preparation is initiated with selection of the sampling locations by the Design Team (ORD in Corvallis). The list of sampling locations is distributed to the EPA Regional Lakes Survey Coordinators, states, and tribes. With the sampling location list, state and tribal field crews can begin site reconnaissance on the primary sites and alternate replacement sites and begin work on obtaining access permission to each site. Specific

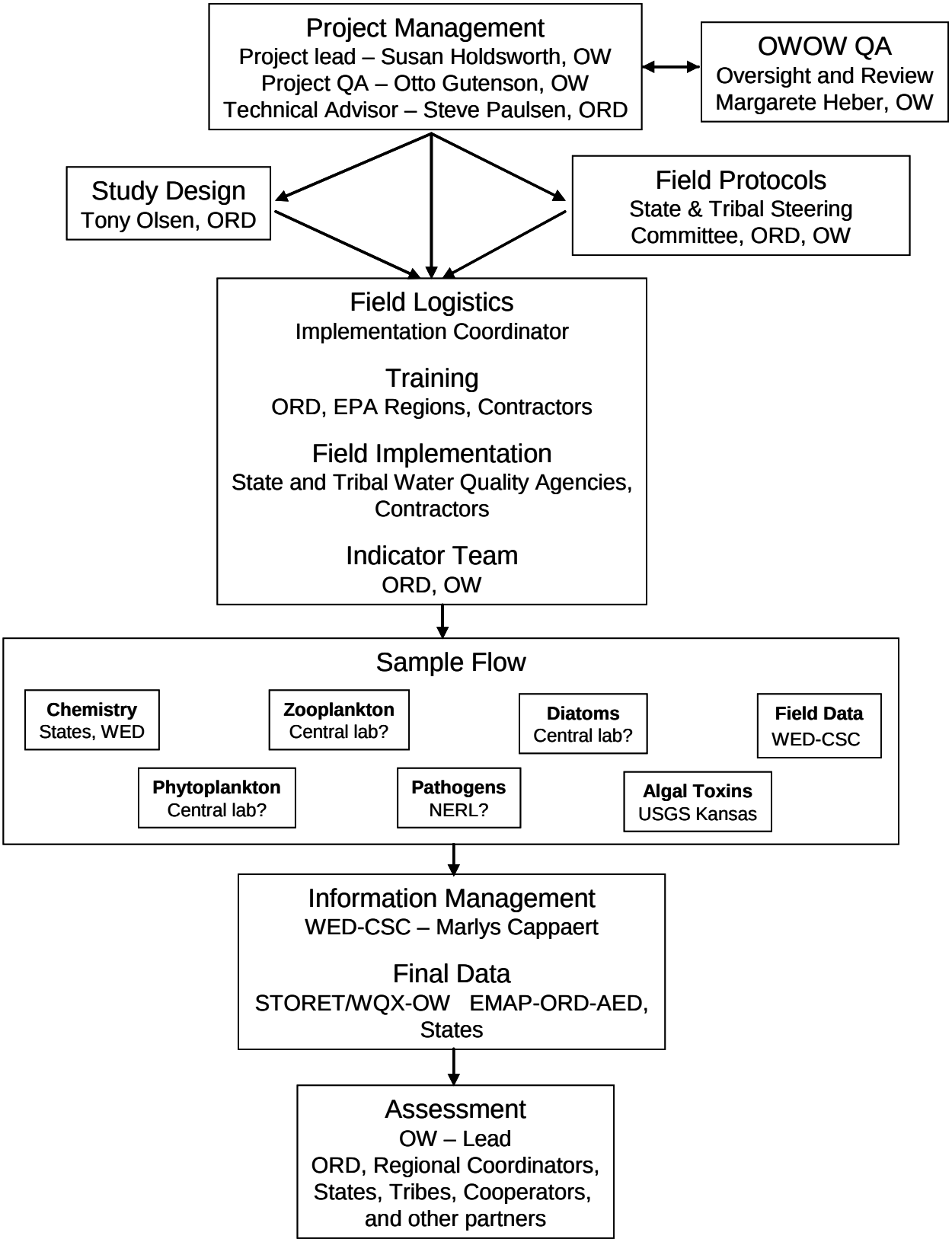

Figure 1-1. Lakes Survey project organization chart.

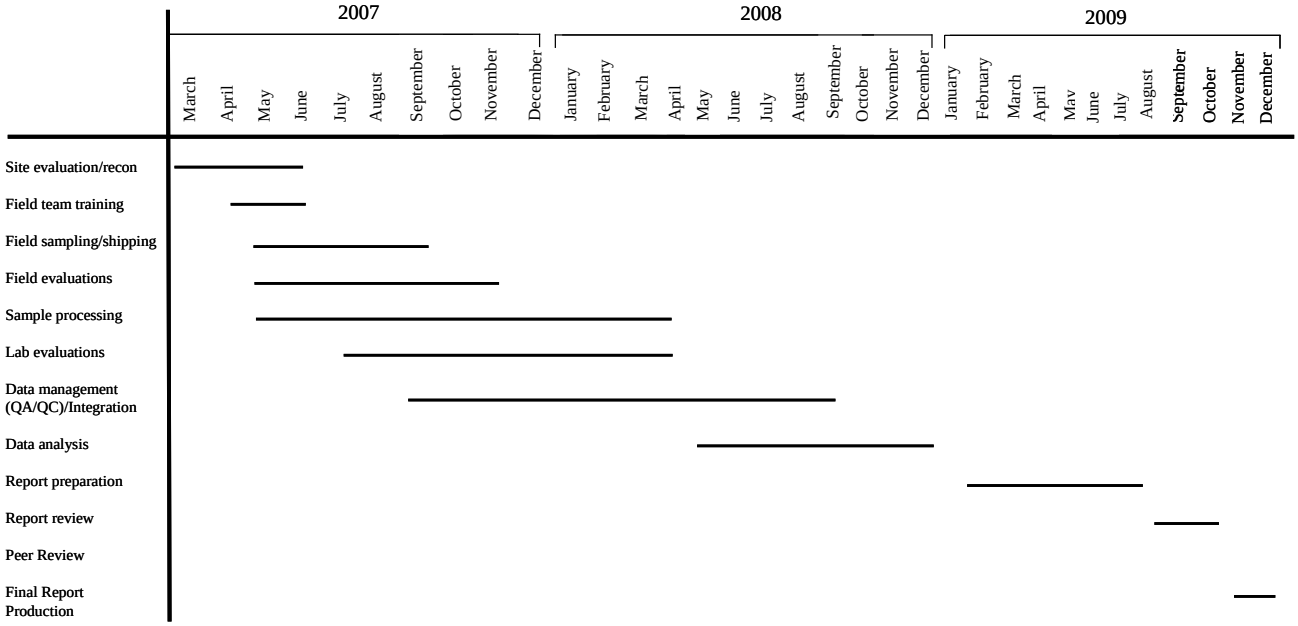

Figure 1-2. Timeline of Lakes Survey project activities.

procedures for evaluating each sampling location and for replacing non-sampleable sites are documented in *Survey of the Nation's Lakes: Site Evaluation Guidelines*. Scientific collecting permits from State and Federal agencies will be procured, as needed. The field teams will use standard field equipment and supplies as identified in the Equipment and Supplies List (Appendix A of the Field Operations Manual). Field Team coordinators from states and tribes will work with EPA Regional Coordinators and the Information Management Center to coordinate equipment and supply requirements. This helps to ensure comparability of protocols across states. Detailed lists of equipment required for each field protocol, as well as guidance on equipment inspection and maintenance, are contained in the Field Operations Manual.

Field measurements and samples are collected by trained teams. The field team leaders must be trained at EPA-sponsored training. Ideally, all members of each field team should attend one EPA-sponsored training session before the field season in their state or tribal jurisdiction. Field sampling audits or evaluation visits will be completed for each field team. The training program stresses hands-on practice of methods, consistency among crews, collection of high quality data and samples, and safety. Training documentation will be maintained by the Project QA Officers.

For each lake, a dossier is prepared and contains the following applicable information: road maps, copies of written access permissions, scientific collection permits, coordinates of lake sites, information brochures on the program for interested land owners, a bathymetric map with the index site location marked (if available), and local area emergency numbers. Whenever possible, field team leaders attempt to contact landowners approximately two days before the planned sampling date. Procedures for land owner notification can be found in the Site Evaluation Guidelines. As the design requires repeat visits to select sampling locations, it is important for the field teams to do everything possible to maintain good relationships with

landowners. This includes prior contacts, respect of special requests, closing gates, minimal site disturbance, and removal of all materials, including trash, associated with the sampling visit.

A variety of methods may be used to access a lake, including vehicles and boats. Some sampling locations require teams to hike in, transporting all equipment in backpacks. For this reason, ruggedness and weight are important considerations in the selection of equipment and instrumentation. Teams may need to camp out at the sampling location and so are equipped with the necessary camping equipment.

The site verification process is shown in Figure 1-3. Upon arrival at a site, the location is verified by a Global Positioning System (GPS) receiver, landmark references, and/or local residents. Samples and measurements for various parameters are collected in a specified order (Figure 1-4). This order has been set up to minimize the impact of sampling for one parameter upon subsequent parameters. All methods are fully documented in step-by-step procedures in the *Survey of the Nation's Lakes: Field Operations Manual* (USEPA 2007). The manual also contains detailed instructions for completing documentation, labeling samples, any field processing requirements, and sample storage and shipping. Field communications will be through Field Team Coordinators, and may involve regularly scheduled conference calls or contacts with the Communications Center.

Standardized field data forms are the primary means of data recording. On completion, the data forms are reviewed by a person other than the person who initially entered the information. Prior to departure from the field site, the field team leader reviews all forms and labels for completeness and legibility and ensures that all samples are properly labeled and packed.

Upon return from field sampling to the office, completed data forms are sent to the information management staff at WED in Corvallis, Oregon for entry into a computerized data base. At WED, electronic data files are reviewed independently to verify that values are consistent with those recorded on the field data form or original field data file (see Section 4.1.4).

Samples are stored or packaged for shipment in accordance with instructions contained in the Field Operations Manual. Precautions are taken so holding times are not exceeded. Samples which must be shipped are delivered to a commercial carrier; copies of bills of lading or other documentation are maintained by the team. The Information management Center is notified to track the sample shipment; thus, tracing procedures can be initiated quickly in the event samples are not received. Chain-of-custody forms are completed for all transfers of samples, with copies maintained by the field team.

The field operations phase is completed with collection of all samples or expiration of the sampling window. These debriefings cover all aspects of the field program and solicit suggestions for improvements.

1.3.2 Overview of Laboratory Operations

Holding times for surface water samples vary with the sample types and analytes. Thus, some analytical measurements begin during sampling (e.g., *in situ* profiles) while others are not initiated until sampling has been completed (e.g., phytoplankton, zooplankton). Analytical

methods are summarized in the specific Field and Laboratory SOPs that are companion documents to this QAPP.

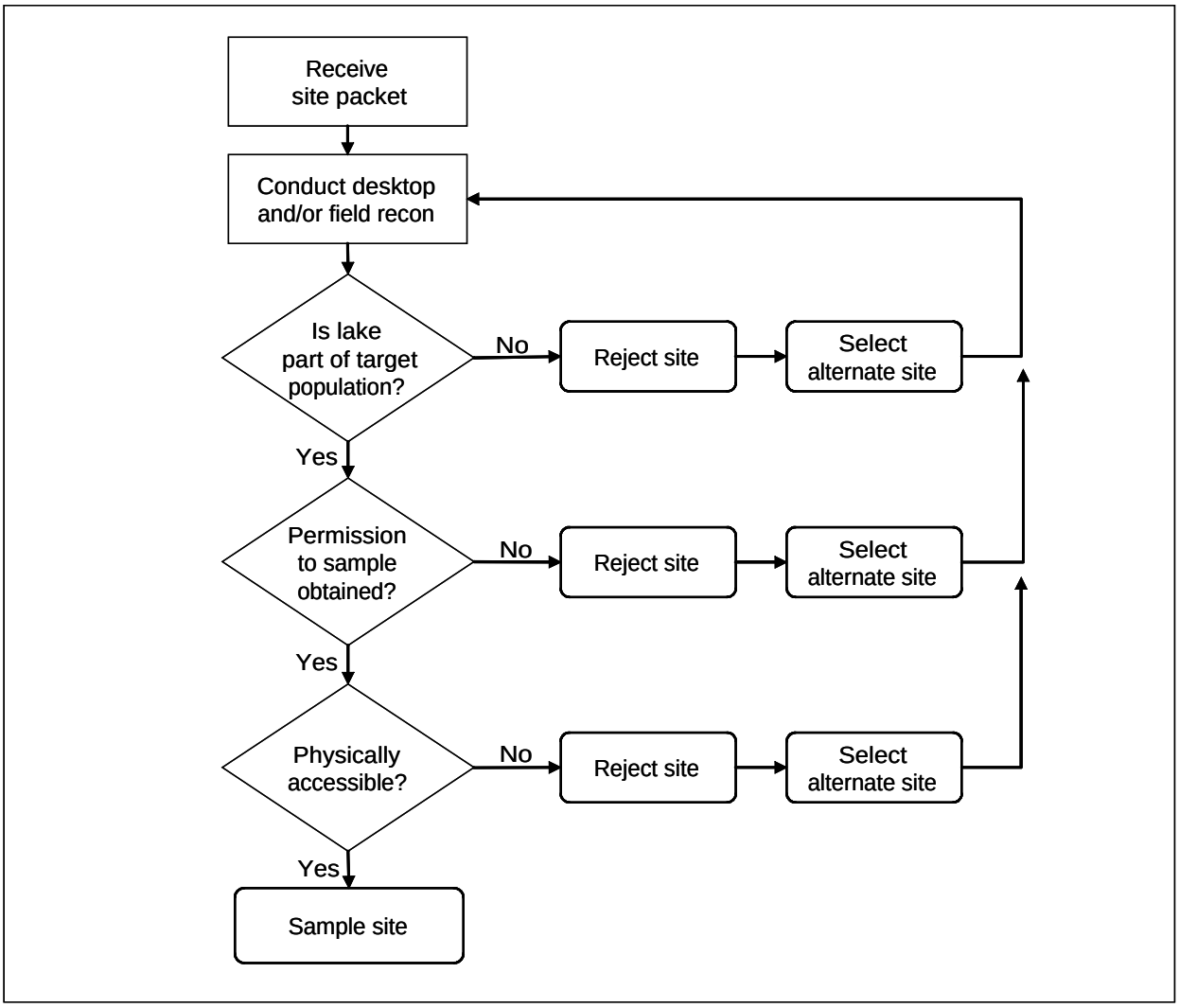

Figure 1-3. Site verification process.

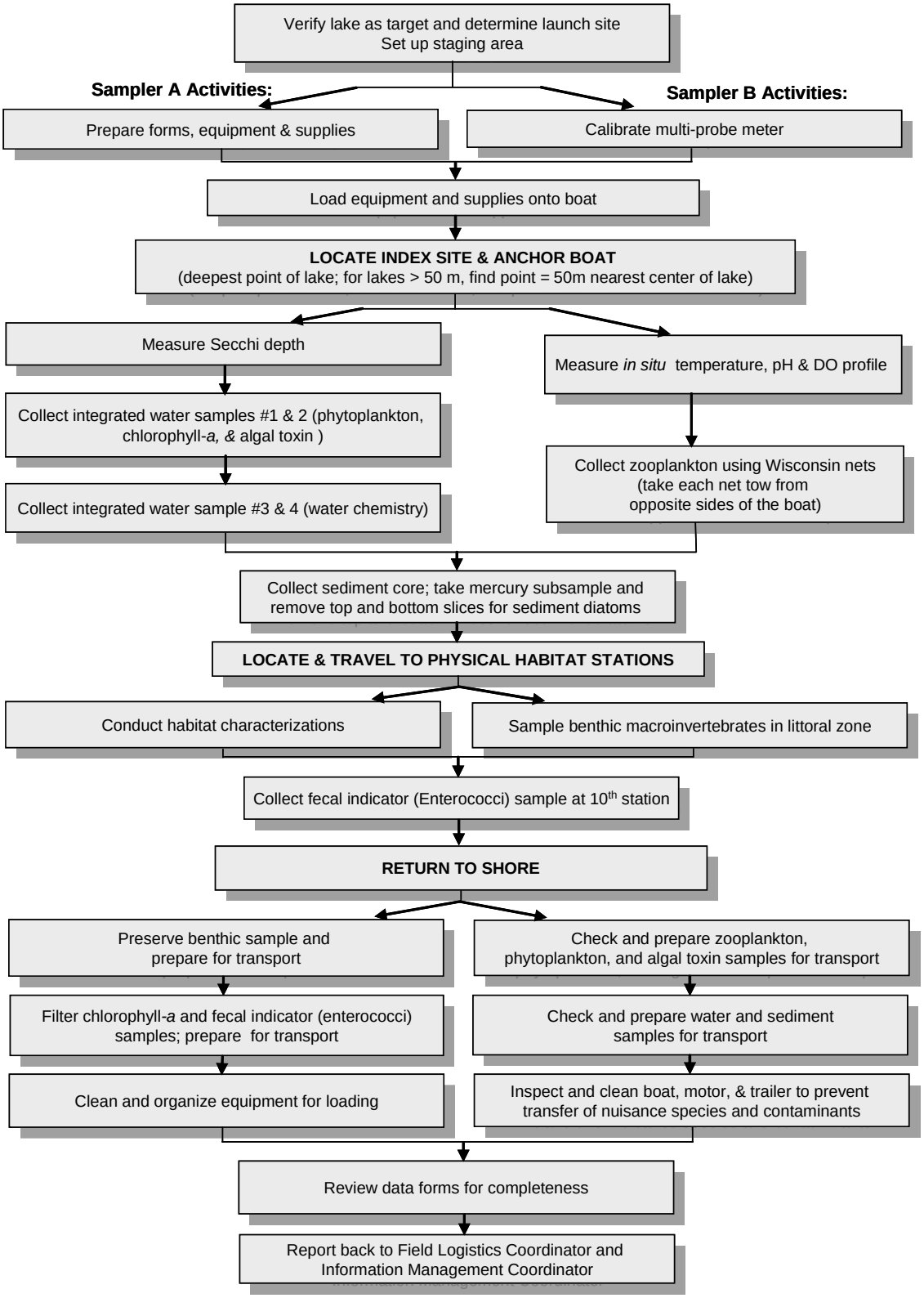

Figure 1-4. Summary of field activities and lake sampling.

Chemical, physical, or biological analyses may be performed in-house or by contractor or cooperator laboratories. Laboratories providing analytical support must have the appropriate facilities to properly store and prepare samples and appropriate instrumentation and staff to provide data of the required quality within the time period dictated by the project. Laboratories are expected to conduct operations using good laboratory practices. General guidelines for analytical support laboratories:

- A program of scheduled maintenance of analytical balances, water purification systems, microscopes, laboratory equipment, and instrumentation.

- Verification of the calibration of analytical balances using class "S" weights which are certified by the National Institute of Standards and Technology (NIST).

- Verification of the calibration of top-loading balances using NIST-certified class "P" weights.

- Checking and recording the composition of fresh calibration standards against the previous lot. Acceptable comparisons are ± 2 percent of the theoretical value. (This acceptance is tighter than the method calibration criteria.)

- Recording all analytical data in bound logbooks in ink, or on standardized recording forms.

- Verification of the calibration of uniquely identified daily use thermometers using NIST-certified thermometers.

- Monitoring and recording (in a logbook or on a recording form) temperatures and performance of cold storage areas and freezer units (where samples, reagents, and standards may be stored). During periods of sample collection operations, monitoring must be done on a daily basis.

- An overall program of laboratory health and safety including periodic inspection and verification of presence and adequacy of first aid and spill kits; verification of presence and performance of safety showers, eyewash stations, and fume hoods; sufficiently exhausted reagent storage units, where applicable; available chemical and hazardous materials inventory; and accessible material safety data sheets for all required materials.

- An overall program of hazardous waste management and minimization, and evidence of proper waste handling and disposal procedures (90-day storage, manifested waste streams, etc.).

- If needed, having a source of reagent water meeting American Society of Testing and Materials (ASTM) Type I specifications for conductivity (< 1 µS/cm at 25 °C; ASTM 1984) available in sufficient quantity to support analytical operations.

- Appropriate microscopes or other magnification for biological sample sorting and organism identification.

- Labeling all containers used in the laboratory with date prepared, contents, and initials of the individual who prepared the contents.

- Dating and storing all chemicals safely upon receipt. Chemicals are disposed of properly when the expiration date has expired.

- Using a laboratory information management system to track the location and status of any sample received for analysis.

- Reporting results using standard formats and units compatible with the information management system.

All laboratories providing analytical support to Lakes Survey must adhere to the provisions of this integrated QAPP. Laboratories will provide information documenting their ability to conduct the analyses with the required level of data quality before analyses begin. The documentation will be sent to Sarah Lehmann at EPA Headquarters. Such information might include results from interlaboratory comparison studies, analysis of performance evaluation samples, control charts and results of internal QC sample or internal reference sample analyses to document achieved precision, bias, accuracy, and method detection limits. Contracted laboratories will be required to provide copies of their Data Management Plan. Laboratory operations may be evaluated by technical systems audits, performance evaluation studies, and by participation in interlaboratory sample exchange.

1.3.3 Data Analysis and Reporting

A technical workgroup convened by the EPA Project Leader is responsible for development of a data analysis plan that includes a verification and validation strategy. These processes are described in the internal indicator research strategies and summarized in the indicator-specific sections of this QAPP. Validated data are transferred to the central data base managed by EMAP information management support staff located at WED in Corvallis. Information management activities are discussed further in Section 4. Data in the WED data base are available to Cooperators for use in development of indicator metrics. All validated measurement and indicator data from the Lakes Survey are eventually transferred to EPA's Water Quality Exchange (WQX) that will replace the STORET data management system.

1.3.4 Peer Review

The Survey will undergo a thorough peer review process, where the scientific community and the public will be given the opportunity to provide comments. Cooperators have been actively involved in the development of the overall project management, design, methods, and standards including the drafting of four key project documents:

- *Quality Assurance Project Plan (EPA 841-B-07-003)*
- *Lake Evaluation Guidelines (EPA 841-B-06-003)*
- *Field Operations Manual (EPA 841-B-07-004)*
- *Laboratory Methods Manual (EPA841-B-07-005)*

Outside scientific experts from universities, research centers, and other federal agencies have been instrumental in indicator development and will continue to play an important role in data analysis.

The EPA will utilize a three tiered approach for peer review of the Survey: (1) internal and external review by EPA, states, other cooperators and partners, (2) external scientific peer review, and (3) public review.

Once data analysis has been completed, cooperators will examine the results at regional meetings. Comments and feedback from the cooperators will be incorporated into the draft report. Public and scientific peer review will happen simultaneously. This public comment period is important to the process and will allow us to garner a broader perspective in examining the results before the final report. The public peer review is consistent with the Agency and OMB's revised requirements for peer review.

Below are the proposed measures EPA will implement for engaging in the peer review process:

1) Develop and maintain a public website with links to standard operating procedures, quality assurance documents, fact sheets, cooperator feedback, and final report
2) Conduct technical workgroup meetings composed of scientific experts, cooperators, and EPA to evaluate and recommend data analysis options and indicators
3) Hold national meeting where cooperators will provide input and guidance on data presentation and an approach for data analysis
4) Complete data validation on all chemical, physical and biological data
5) Conduct final data analysis with workgroup to generate assessment results
6) Engage peer review contractor to identify external peer review panel
7) Develop draft report presenting assessment results
8) Conduct regional meetings with cooperators to examine and comment on results
9) Develop final draft report incorporating input from cooperators and results from data analysis group to be distributed for peer and public review
10) Issue Federal Register (FR) Notice announcing document availability and hold scientific/peer review and public comment (30-45 days)
11) Consider scientific and public comments and produce a final report

The proposed peer review schedule is provided below and is contingent upon timeliness of data validation, schedule availability for regional meetings and experts for data analysis workshop.

May 2008 - December 2008	Data validation
March 15, 2009	Data analysis workshop
May - August 2009	Internal peer review meetings with states, cooperators, participants
October 19, 2009	Release for external peer and public review of draft

2.0 DATA QUALITY OBJECTIVES

It is a policy of the U.S. EPA that Data Quality Objectives (DQOs) be developed for all environmental data collection activities following the prescribed DQO Process. DQOs are qualitative and quantitative statements that clarify study objectives, define the appropriate types of data, and specify the tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions (EPA 2006). Data

quality objectives thus provide the criteria to design a sampling program within cost and resource constraints or technology limitations imposed upon a project or study. DQOs are typically expressed in terms of acceptable uncertainty (e.g., width of an uncertainty band or interval) associated with a point estimate at a desired level of statistical confidence (EPA 2006). The DQO Process is used to establish performance or acceptance criteria, which serve as the basis for designing a plan for collecting data of sufficient quality and quantity to support the goals of a study (EPA 2006). As a general rule, performance criteria represent the full set of specifications that are needed to design a data or information collection effort such that, when implemented, generate newly-collected data that are of sufficient quality and quantity to address the project's goals (EPA 2006). Acceptance criteria are specifications intended to evaluate the adequacy of one or more existing sources of information or data as being acceptable to support the project's intended use (EPA 2006).

2.1 Data Quality Objectives for Lakes Survey

Target DQOs established for the Lakes Survey relate to the goal of describing the current status in the condition of selected indicators of the condition of lakes in the conterminous U.S. and ecoregions of interest. The formal statement of the DQO for national estimates is as follows:

- Estimate the proportion of lakes (± 5%) in the conterminous U.S. that falls below the designated threshold for good conditions for selected measures with 95% confidence.

For the ecoregions of interest the DQO is:

- Estimate the proportion of lakes (± 15%) in a specific ecoregion that fall below the designated threshold for good conditions for selected measures with 95% confidence.

2.2 Measurement Quality Objectives

For each parameter, performance objectives (associated primarily with measurement error) are established for several different data quality indicators (following USEPA Guidance for Quality Assurance Plans EPA240/R-02/009). Specific measurement quality objectives (MQOs) for each parameter are presented in the indicator section of this QAPP. The following sections define the data quality indicators and present approaches for evaluating them against acceptance criteria established for the program.

2.2.1 Laboratory Reporting Level (Sensitivity)

For chemical measurements, requirements for the method detection limit (MDL) are typically established. The MDL is defined as the lowest level of analyte that can be distinguished from zero with 99 percent confidence based on a single measurement (Glaser et al., 1981). USGS NWQL has developed a variant of the MDL called the long-term MDL (LT-MDL) to capture greater method variability (Oblinger Childress et al. 1999). Unlike MDL, it is designed to incorporate more of the measurement variability that is typical for routine analyses in a production laboratory, such as multiple instruments, operators, calibrations, and sample preparation events (Oblinger Childress et al. 1999). The LT-MDL determination ideally employs at least 24 spiked samples prepared and analyzed by multiple analysts on multiple instruments over a 6- to 12-month period at a frequency of about two samples per month (EPA 2004). The

LT-MDL uses "F-pseudosigma" (Fσ) in place of *s*, the sample standard deviation, used in the EPA MDL calculation. F-pseudosigma is a non-parametric measure of variability that is based on the interquartile range of the data (EPA 2004). The LT-MDL may be calculated using either the mean or median of a set of long-term blanks, or from long-term spiked sample results (depending o the analyte and specific analytical method). The LT-MDL for an individual analyte is calculated as:

Equation 1a

$$LT\text{-}MDL = M + (t_{0.99,n-1} \times F_\sigma)$$

where *M* is the mean or median of blank results; *n* is the number of spiked sample results; and *FΦ* is F-pseudosigma, a nonparametric estimate of variability calculated as:

Equation 1b

$$F_\sigma = \frac{Q_3 - Q_1}{1.349}$$

where: *Q3* and *Q1* are the 75th percentile and 25th percentile of spiked sample results, respectively.

LT-MDL is designed to be used in conjunction with a laboratory reporting level (LRL; Oblinger Childress et al. 1999). The LRL is designed to achieve a risk of ≤1% for both false negatives and false positives (Oblinger Childress et al. 1999). The LRL is set as a multiple of the LT-MDL, and is calculated as follows:

$$LRL = 2 \times LT\text{–}MDL$$

Therefore, multiple measurements of a sample having a true concentration at the LRL should result in the concentration being detected and reported 99 percent of the time (Oblinger Childress et al. 1999).

All laboratories will develop calibration curves for each batch of samples that include a calibration standard with an analyte concentration equal to the LRL. Estimates of LRLs (and how they are determined) are required to be submitted with analytical results. Analytical results associated with LRLs that exceed the objectives are flagged as being associated with unacceptable LRLs. Analytical data that are below the estimated LRLs are reported, but are flagged as being below the LRLs.

2.2.2 Precision, Bias, and Accuracy

Precision and bias are estimates of random and systematic error in a measurement process (Kirchmer, 1983; Hunt and Wilson, 1986, USEPA 2002). Collectively, precision and bias provide an estimate of the total error or uncertainty associated with an individual measurement or set of measurements. Systematic errors are minimized by using validated methods and standardized procedures across all laboratories. Precision is estimated from repeated measurements of samples. Net bias is determined from repeated measurements of solutions of known composition, or from the analysis of samples that have been fortified by the addition of a known quantity of analyte. For analytes with large ranges of expected

concentrations, MQOs for precision and bias are established in both absolute and relative terms, following the approach outlined in Hunt and Wilson (1986). At lower concentrations, MQOs are specified in absolute terms. At higher concentrations, MQOs are stated in relative terms. The point of transition between an absolute and relative MQO is calculated as the quotient of the absolute objective divided by the relative objective (expressed as a proportion, e.g., 0.10 rather than as a percentage, e.g., 10%).

Precision in absolute terms is estimated as the sample standard deviation when the number of measurements is greater than two:

Equation 1

$$s = \sqrt{\frac{i = \sum_{1}^{n} (xi - \bar{x})^2}{n-1}}$$

where x_i is the value of the replicate, $\bar{x}$ is the mean of repeated sample measurements, and *n* is the number of replicates. Relative precision for such measurements is estimated as the relative standard deviation (RSD, or coefficient of variation, [CV]):

Equation 2

$$RSD = \frac{s}{\bar{X}} \times 100$$

here *s* is the sample standard deviation of the set of measurements, and $\bar{x}$ equals the

mean value for the set of measurements.

Precision based on duplicate measurements is estimated based on the range of measured values (which equals the difference for two measurements). The relative percent difference (RPD) is calculated as:

Equation 3

$$RPD = \left(\frac{|A - B|}{(A + B)/2} \right) \times 100$$

where *A* is the first measured value, *B* is the second measured value.

For repeated measurements of samples of known composition, net bias (*B*) is estimated in absolute terms as:

Equation 4

$$B = \bar{x} - T$$

where $\bar{x}$ equals the mean value for the set of measurements, and *T* equals the theoretical or target value of a performance evaluation sample. Bias in relative terms (*B[%]*) is calculated as:

Equation 5

$$B(\%) = \frac{\bar{x} - T}{T} \times 100$$

where $\bar{x}$ equals the mean value for the set of measurements, and *T* equals the theoretical or target value of a performance evaluation sample.

Accuracy is estimated for some analytes from fortified or spiked samples as the percent recovery. Percent recovery is calculated as:

Equation 6

$$\%recovery = \frac{C_{is} - C_{ii}}{C_s} \times 100$$

where C_{is} is the measured concentration of the spiked sample, C_{ii} is the concentration of the unspiked sample, and C_s is the concentration of the spike.

Precision and bias within each laboratory are monitored for every sample batch by the analysis of internal QC samples. Samples associated with unacceptable QC sample results are reviewed and re-analyzed if necessary. Precision and bias across all laboratories will be evaluated after analyses are completed by using the results of performance evaluation (PE) samples sent to all laboratories (3 sets of 3 PE samples, with each set consisting of a low, moderate, and high concentration sample of all analytes).

2.2.3 Taxonomic Precision and Accuracy of Benthic Macroinvertebrates

For the Lakes Survey, taxonomic precision will be quantified by comparing whole-sample identifications completed by independent taxonomists or laboratories. Accuracy of taxonomy will be qualitatively evaluated through specification of target hierarchical levels (e.g., family, genus, or species); and the specification of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). To calculate taxonomic precision, 10 percent of the samples will be randomly-selected for re-identification by an independent, outside taxonomist or laboratory. Comparison of the results of whole sample re-identifications will provide a Percent Taxonomic Disagreement (PTD) calculated as:

Equation 7

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

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 are taxonomic results and the overall taxonomic precision is better. A MQO of 15% 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.

Where re-identification by an independent, outside taxonomist or laboratory is not practical for benthic macroinvertebrates, percent similarity will be calculated. Percent similarity is a measure of similarity between two communities or two samples (Washington 1984). Values range from 0% for samples with no species in common, to 100% for samples which are identical. It is calculated as follows:

Equation 8

$$PSC = 1 - 0.5\sum_{i=1}^{K}\left|a - b\right|$$

where: *a* and *b* are, for a given species, the relative proportions of the total samples A and B, respectively, which that species represents. A MQO of ≥85% is recommended for percent similarity of taxonomic identification. If the MQO is not met, the reasons for the discrepancies between analysts should be discussed. If a major discrepency is found in how the two analysts have been identifying organisms, the last batch of samples that have been counted by the analyst under review may have to be recounted.

Additionally, percent similarity should be calculated for re-processed subsamples. This provides a quantifiable measure of the precision of subsampling procedures employed for benthic macroinvertebrates. A MQO of ≥70% is recommended for percent similarity of subsamples. If a sample does not meet this threshold, additional subsamples should be processed from that sample until the MQO is achieved.

Sample enumeration is another component of taxonomic precision. Final specimen counts for samples are dependent on the taxonomist, not the rough counts obtained during the sorting activity. Comparison of counts is quantified by calculation of percent difference in enumeration (PDE), calculated as:

Equation 9

$$PDE = \left(\frac{\left|Lab1 - Lab2\right|}{Lab1 + Lab2}\right) \times 100$$

An MQO of 5% is recommended (overall mean of ≤5% is acceptable). Individual samples exceeding 5% are examined to determine reasons for the exceedance.

Corrective actions for samples exceeding these MQOs can include defining the taxa for which re-identification may be necessary (potentially even by third party), for which samples (even outside of the 10% lot of QC samples) it is necessary, and where there may be issues of nomenclatural or enumeration problems.

Taxonomic accuracy is evaluated by having individual specimens representative of selected taxa identified by recognized experts. Samples will be identified using the most appropriate technical literature that is accepted by the taxonomic discipline and reflects the accepted nomenclature. Where necessary, the Integrated Taxonomic Information System (ITIS, http://www.itis.usda.gov/) will be used to verify nomenclatural validity and spelling. A reference collection will be compiled as the samples are identified. Specialists in several taxonomic groups will verify selected individuals of different taxa, as determined by the Lakes Survey workgroup.

2.2.4 Completeness

Completeness requirements are established and evaluated from two perspectives. First, valid data for individual parameters must be acquired from a minimum number of sampling locations in order to make subpopulation estimates with a specified level of confidence or sampling precision. The objective of this study is to complete sampling at 95% or more of the 1000 initial sampling sites. Percent completeness is calculated as:

Equation 10

$$\%C = V/T \times 100$$

where *V* is the number of measurements/samples judged valid, and *T* is the total number of planned measurements/samples. Within each indicator, completeness objectives are also established for individual samples or individual measurement variables or analytes. These objectives are estimated as the percentage of valid data obtained versus the amount of data expected based on the number of samples collected or number of measurements conducted. Where necessary, supplementary objectives for completeness are presented in the indicator-specific sections of this QAPP.

The completeness objectives are established for each measurement *per site type* (e.g., probability sites, revisit sites, etc.). Failure to achieve the minimum requirements for a particular site type results in regional population estimates having wider confidence intervals. Failure to achieve requirements for repeat sampling (10% of samples collected) and revisit samples (10% of sites visited) reduces the precision of estimates of index period and annual variance components, and may impact the representativeness of these estimates because of possible bias in the set of measurements obtained.

2.2.5 Comparability

Comparability is defined as the confidence with which one data set can be compared to another (USEPA 2002). A performance-based methods approach is being utilized for water chemistry and chlorophyll-*a* analyses that defines a set of laboratory method performance requirements for data quality. Following this approach, participating laboratories may choose which analytical methods they will use for each target analyte as long as they are able to achieve the performance requirements as listed in Table 5-1. For all parameters, comparability is addressed by the use of standardized sampling procedures and analytical methods by all sampling crews and laboratories. Comparability of data within and among parameters is also facilitated by the implementation of standardized quality assurance and quality control techniques and standardized performance and acceptance criteria. For all measurements, reporting units and format are specified, incorporated into standardized data recording forms, and documented in the information management system. Comparability is also addressed by providing results of QA sample data, such as estimates of precision and bias, conducting methods comparison studies when requested by the grantees and conducting interlaboratory performance evaluation studies among state, university, and Lakes Survey contract laboratories.

2.2.6 Representativeness

Representativeness is defined as "the degree to which the data accurately and precisely represent a characteristic of a population parameter, variation of a property, a process characteristic, or an operational condition" (USEPA 2002). At one level, representativeness is affected by problems in any or all of the other data quality indicators.

At another level, representativeness is affected by the selection of the target surface water bodies, the location of sampling sites within that body, the time period when samples are collected, and the time period when samples are analyzed. The probability-based sampling design should provide estimates of condition of surface water resource populations that are representative of the region. The individual sampling programs defined for each indicator

attempt to address representativeness within the constraints of the *response design*, (which includes when, where, and how to collect a sample at each site). Holding time requirements for analyses ensure analytical results are representative of conditions at the time of sampling. Use of duplicate (repeat) samples which are similar in composition to samples being measured provides estimates of precision and bias that are applicable to sample measurements.

3.0 SAMPLING DESIGN AND SITE SELECTION

The overall sampling program for the Lakes Survey project requires a randomized, probability-based approach for selecting lakes where sampling activities are to be conducted. Details regarding the specific application of the probability design to surface waters resources are described in Paulsen et al. (1991) and Stevens (1994). The specific details for the collection of samples associated with different indicators are described in the indicator-specific sections of this QAPP.

3.1 Probability Based Sampling Design and Site Selection

The target population for this project includes all lakes, reservoirs, and ponds within the 48 contiguous United States greater than 4 hectares (10 acres) in surface area that are permanent waterbodies. Lakes that are saline are excluded as are those used for aquaculture, disposal-tailings, sewage treatment, evaporation, or other unspecified disposal use. The National Hydrography Dataset (NHD) was employed to derive a list of lakes for potential inclusion into the survey. The overall sample size was set to include 1000 lake sampling events, of which 909 are discrete lake samples and 91 are revisits.

A Generalized Random Tessellation Stratified (GRTS) survey design for a finite resource was used for site selection. The design was developed to include a representative subset of the lakes that were sampled in EPA's National Lake Eutrophication Study (NES), which will allow for an extrapolation of changes to the full set of NES lakes. Lake selection for the survey provided for five size class categories (4-10 ha, 10-20 ha, 20-50 ha, 50-100 ha, >100 ha), as well as spatial distribution across the lower 48 states and nine aggregated Omernik Level 3 ecoregions. Small lakes (1-4 ha in size) were also included in the selection process so that states may elect to include these smaller lakes in state-level assessment efforts. An additional 4000 lakes were selected as potential replacement lakes (oversample sites). The oversample is used to replace a candidate lake that is determined to be non-target or to replace a target lake that is not accessible due to landowner denials, physical barriers, or safety concerns. Replacement sites should be taken from the Oversample list in order.

Lakes were selected using a two-stage process employing a systematic grid of sampling points developed for use by all EMAP resource groups (Overton et al. 1991). The selection process is automated, using digital maps and geographic information system (GIS) techniques and equipment (Selle et al. 1991).

QA for GIS methods is focused on aspects of accuracy (e.g., how well do digitized maps provide information of what is actually present at a location) and the representativeness of this information. Three basic types of errors have been identified by the EMAP design group:

- Map-related errors: These are errors due to inconsistencies between different types (or scales) of maps (e.g., paper maps versus digitized versions).

- Landscape-related errors: These are errors due to changes occurring at a site since the corresponding map was last revised. Such changes could be natural (due to natural successional processes) or anthropogenic (e.g., draining a manmade lake or reservoir).

- Other errors: Software developed for digitizing maps or other associated GIS processing applications may introduce errors.

The GIS staff at WED that support surface waters research in EMAP have developed QC procedures for controlling some of these errors. Other types of errors are quantified as they are discovered, essentially by using ground truthing as a standard for comparison.

The first stage of the probability sample (termed the "Tier I" sample) is developed by intersecting the spatial file of surface water body information with a second file containing spatial information related to the EMAP systematic sampling grid. This information includes locational information regarding the sampling points on the grid and an associated 40-km^2 hexagon area centered on each sampling point. The Tier I sample represents all surface water bodies whose digitized labeling points are located within the boundaries of one of the hexagons.

A QC check is made by comparing a selected subset of the Tier I sample against the parent DLGs. Any noted discrepancies are reconciled by using the corresponding paper topographic maps. Error rates for the frame are extrapolated from the error rates found in the Tier I sample.

The second stage of site selection involves selecting a subset of the Tier I sample. This subset (termed the "Tier II" sample), represents sites that are expected to be visited by field sampling crews. The Tier II sample is selected through a process that incorporates the desired Tier II sample size stratified into multi-density categories. Sites are selected randomly from the Tier I sample, with the constraint that the spatial distribution of sites be preserved. Each Tier II site has an associated inclusion probability with which any measured attribute can be related to the target population of sites.

Revisit Sites: Of the sites visited in the field and found to be target sites, a total of 10% will be revisited. The primary purpose of this revisit set of sites is to allow variance estimates that would provide information on the extent to which the population estimates might vary if they were sampled at a different time.

Oversample Lake Sites: The number of sites that must be evaluated to achieve the expected number of field sites that can be sampled can only be estimated based on assumptions concerning expected error rates in NHD, percent of landowner refusals, and percent of physically inaccessible sites. Based on the estimates gained in previous studies, a list of 4000 alternate sites was selected at the same time as the base sites. The large oversample size was done primarily to accommodate those states who may want to increase the number of lakes sampled within their state for a state-level design. Alternate sites must be used in order until the desired sample size has been achieved.

4.0 INFORMATION MANAGEMENT

Like QA, information management (IM) is integral to all aspects of the Lakes Survey from initial selection of sampling sites through dissemination and reporting of final, validated data. QA and QC measures implemented for the IM system are aimed at preventing corruption of data at the time of their initial incorporation into the system and maintaining the integrity of data and information after incorporation into the system. The general organization of, and QA/QC measures associated with, the IM systems are described in this section.

4.1 Overview of System Structure

At each point where data and information are generated, compiled, or stored, the information must be managed. Thus, the IM system includes all of the data-generating activities, all of the means of recording and storing information, and all of the processes which use data. The IM system includes both hardcopy and electronic means of generating, storing, and archiving data. All participants in the Lakes Survey have certain responsibilities and obligations which make them a part of the IM system. In its entirety, the IM system includes site selection and logistics information, sample labels and field data forms, tracking records, map and analytical data, data validation and analysis processes, reports, and archives. IM staff supporting the Lakes Survey at WED provides support and guidance to all program operations in addition to maintaining a central data base management system for the Lakes Survey data.

The central repository for data and associated information collected for use by the Lakes Survey is a secure, access-controlled server located at WED-Corvallis. The general organization of the information management system is presented in Figure 4-1. Data are stored and managed on this system using the Statistical Analysis System (SAS) software package. This centrally managed IM system is the primary data management center for the Lakes Survey research conducted at WED and elsewhere. The IM staff receives, enters, and maintains data and information generated by the site selection process (see Section 3), field sample and data collection, map-based measurements, laboratory analyses, and verification and validation activities completed by the indicator leads. In addition to this inflow, the IM system provides outflow in provision of data files to Lakes Survey staff and other users. The IM staff at WED is responsible for maintaining the security integrity of both the data and the system.

The following sections describe the major inputs to the central data base and the associated QA/QC processes used to record, enter, and validate measurement and analytical data collected for EMAP surface waters research projects. Activities to maintain the integrity and assure the quality of the contents of the IM system are also described.

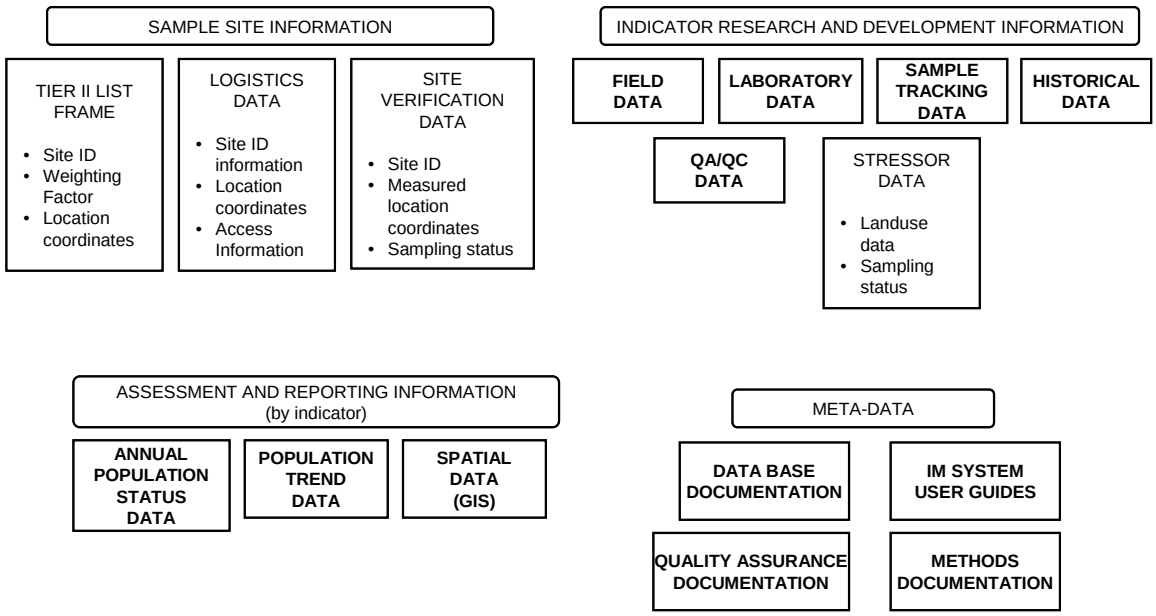

Figure 4-1. Organization of information management system modeled after EMAP Surface Water Information Management (SWIM) system for the Lakes Survey.

4.1.1 Design and Site Status Data Files

The site selection process described in Section 3 produces a list of candidate sampling locations, inclusion probabilities, and associated site classification data (e.g., target status, ecoregion, etc.). This "design" data file is provided to the IM staff, implementation coordinators, and field coordinators. Field coordinators determine ownership and contacts for acquiring permission to access each site, and conduct site evaluation and reconnaissance activities. Ownership, site evaluation, and reconnaissance information for each site are compiled into a "site status" data file. Generally, standardized forms are used during reconnaissance activities. Information from these forms may be entered into a SAS compatible data management system. Whether in electronic or hardcopy format, a copy of the logistics data base is provided to the IM for archival.

4.1.2 Sample Collection and Field Data Recording

Prior to initiation of field activities, the IM staff works with the indicator leads and analytical support laboratories to develop standardized field data forms and sample labels. Preprinted adhesive labels having a standard recording format are completed and affixed to each sample container. Precautions are taken to ensure that label information remains legible and the label remains attached to the sample. Examples of sample labels are presented in the Field Operations Manual.

Field sample collection and data forms are designed in conjunction with IM staff to ensure the format facilitates field recording and subsequent data entry tasks. All forms which may be used onsite are printed on water-resistant paper. Copies of the field data forms and instructions for completing each form are documented in the Field Operations Manuals. Recorded data are reviewed upon completion of data collection and recording activities by a person other than the one who completed the form. Field crews check completed data forms

and sample labels before leaving a sampling site to ensure information and data were recorded legibly and completely. Errors are corrected if possible, and data considered as suspect are qualified using a flag variable. The field sampling crew enters explanations for all flagged data in a comments section. Completed field data forms are transmitted to the IM staff at WED for entry into the central data base management system; indicator leads also receive copies of all field-recorded data.

If portable PCs (or handheld data recorders) are to be used in the field, user screens are developed that duplicate the standardized form to facilitate data entry. Specific output formats are available to print data for review and for production of shipping forms. Data may be transferred via modem on a daily basis. Each week CDs containing all down-loaded data for the week are mailed to the IMC.

All samples are tracked from the point of collection. If field PCs are used, tracking records are generated by custom-designed software. Hardcopy tracking and custody forms are completed if PCs are not available for use. Copies of the shipping and custody record accompany all sample transfers; other copies are transmitted to the IMC and applicable indicator lead. Samples are tracked to ensure that they are delivered to the appropriate laboratory, that lost shipments can be quickly identified and traced, and that any problems with samples observed when received at the laboratory are reported promptly so that corrective action can be taken if necessary. Detailed procedures on shipping and sample tracking can be found in the Field Operations Manual.

Procedures for completion of sample labels and field data forms, and use of PCs are covered extensively in training sessions. General QC checks and procedures associated with sample collection and transfer, field measurements, and field data form completion for most indicators are listed in Table 4-1. Additional QA/QC checks or procedures specific to individual indicators are described in the indicator sections in Section 5 of this QAPP.

4.1.3 Laboratory Analyses and Data Recording

Upon receipt of a sample shipment, analytical laboratory receiving personnel check the condition and identification of each sample against the sample tracking record. Each sample is identified by information written on the sample label and by a barcode label. Any discrepancies, damaged samples, or missing samples are reported to the IM staff and indicator lead by telephone.

Most of the laboratory analyses for the Lakes Survey indicators, particularly chemical and physical analyses, follow or are based on standard methods. Standard methods generally include requirements for QC checks and procedures. General laboratory QA/QC procedures applicable to most Lakes Survey indicators are described in Table 4-2. Additional QA/QC samples and procedures specific to individual indicator analyses are described in the indicator sections of this QAPP. Biological sample analyses are generally based on current acceptable practices within the particular biological discipline. Some QC checks and procedures applicable to most Lakes Survey biological samples are described in Table 4-3. Additional QA/QC procedures specific to individual parameters are described in the indicator section of this QAPP.

Table 4-1. Sample and field data quality control activities

Quality Control Activity	Description and/or Requirements
Contamination Prevention	All containers for individual site sealed in plastic bags until use; specific contamination avoidance measures covered in training
Sample Identification	Pre-printed labels with unique ID number on each sample
Data Recording	Data recorded on pre-printed forms of water-resistant paper; field sampling crew reviews data forms for accuracy, completeness, and legibility
Data Qualifiers	Defined qualifier codes used on data form; qualifiers explained in comments section on data form
Sample Custody	Unique sample ID and tracking form information entered in LIMS; sample shipment and receipt confirmed
Sample Tracking	Sample condition inspected upon receipt and noted on tracking form with copies sent to Indicator Lead and/or IM
Data Entry	Data entered using customized entry screens that resemble the data forms; entries reviewed manually or by automated comparison of double entry
Data Submission	Standard format defined for each measurement including units, significant figures, and decimal places, accepted code values, and required field width
Data Archival	All data records, including raw data, archived in an organized manner in compliance with EPA and Federal Government records management policies. Processed samples and reference collections of taxonomic specimens submitted for cataloging and curation at an appropriate museum facility

Table 4-2. Laboratory data quality control activities

Quality Control Activity	Description and/or Requirements
Instrument Maintenance	Follow manufacturer's recommendations and specific guidelines in methods; maintain logbook of maintenance/repair activities
Calibration	Calibrate according to manufacturer's recommendations and guidelines given in Section 5.1.5; recalibrate or replace before analyzing any samples
QC Data	Maintain control charts, determine LT-MDLs and achieved data attributes; include QC data summary (narrative and compatible electronic format) in submission package
Data Recording	Use software compatible with EMAP-SWIM system; check all data entered against the original bench sheet to identify and correct entry errors. Review other QA data (e.g. condition upon receipt, etc.) for possible problems with sample or specimens.
Data Qualifiers	Use defined qualifier codes; explain all qualifiers
Data Entry	Automated comparison of double entry or 100% manual check against original data form
Submission Package	Includes: Letter by the laboratory manager; data, data qualifiers and explanations; electronic format compatible with EMAP-SWIM system, documentation of file and data base structures, variable descriptions and formats; summary report of any problems and corrective actions implemented

Table 4-3. Biological sample quality control activities

Quality Control Activity	Description and/or Requirements
Taxonomic Nomenclature	Use accepted common and scientific nomenclature and unique entry codes
Taxonomic Identifications	Use standard taxonomic references and keys; maintain bibliography of all references used
Independent Identifications	Uncertain identifications to be confirmed by expert in particular taxa
Duplicate Identifications	At least 5% of all samples completed per taxonomist reidentified by different analyst; less than 10% assigned different ID
Taxonomic Reasonableness Checks	Species or genera known to occur in given conditions or geographic area
Reference Collections	Permanent mounts or voucher specimens of all taxa encountered

A laboratory's IM system may consist of only hardcopy records such as bench sheets and logbooks, an electronic laboratory information management system (LIMS), or some combination of hardcopy and electronic records. Laboratory data records are reviewed at the end of each analysis day by the designated laboratory onsite QA coordinator or by supervisory personnel. Errors are corrected if possible, and data considered as suspect by laboratory analysts are qualified with a flag variable. All flagged data are explained in a comments section. Private contract laboratories generally have a laboratory quality assurance plan and established procedures for recording, reviewing, and validating analysis data.

Once analytical data have passed all of the laboratory's internal review procedures, a submission package is prepared and transferred to the IM staff. The contents of the submission package are largely dictated by the type of analysis (physical, chemical, or biological), but generally includes at least the elements listed in the Field and Laboratory Operations Manuals.

Remaining sample material and voucher specimens may be transferred to EPA's designated laboratory or facilities as directed by the EPA Project Leader. All samples and raw data files (including logbooks, bench sheets, and instrument tracings) are to be retained permanently 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.)

4.1.4 Data Review, Verification, and Validation Activities

Raw data files are created from entry of field and analytical data, including data for QA/QC samples and any data qualifiers noted on the field forms or analytical data package. After initial entry, data are reviewed for entry errors by either a manual comparison of a printout of the entered data against the original data form or by automated comparison of data entered twice into separate files. Entry errors are corrected and reentered. For biological samples, species identifications are corrected for entry errors associated with incorrect or misspelled codes. Errors associated with misidentification of specimens are corrected after voucher specimens have been confirmed and the results are available. Files corrected for entry errors are considered to be raw data files. Copies of all raw data files are maintained in the centralized IM system.

The Tetra Tech facilitation team will work with Indicator Leads and the IM (primary data recipients) to ensure that sufficient QC activities are engaged in the various data management

processes. A copy of the raw data files are maintained in the central IM system, generally in active files until completion of reporting and then in archive files. Redundant copies are maintained of all data files and all files are periodically backed up.

Some of the typical checks made in the processes of verification and validation are described in Table 4-4. Automated review procedures may be used. The primary purpose of the initial checks is to confirm that a data value present in an electronic data file is accurate with respect to the value that was initially recorded on a data form or obtained from an analytical instrument. In general, these activities focus on individual variables in the raw data file and may include range checks for numeric variables, frequency tabulations of coded or alphanumeric variables to identify erroneous codes or misspelled entries, and summations of variables reported in terms of percent or percentiles. In addition, associated QA information (e.g., sample holding time) and QC sample data are reviewed to determine if they meet acceptance criteria. Suspect values are assigned a data qualifier. They will either be corrected, replaced with a new acceptable value from sample reanalysis, or confirmed suspect after sample reanalysis. Any suspect data will be flagged for data qualification.

Table 4-4. Data review, verification, and validation quality control activities

Quality Control Activity	Description and/or Requirements
Review any qualifiers associated with variable	Determine if value is suspect or invalid; assign validation qualifiers as appropriate
Summarize and review replicate sample data	Identify replicate samples with large variance; determine if analytical error or visit-specific phenomenon is responsible
Determine if MQOs and project DQOs have been achieved	Determine potential impact on achieving research and/or program objectives
Exploratory data analyses (univariate, bivariate, multivariate) utilizing all data	Identify outlier values and determine if analytical error or site-specific phenomenon is responsible
Confirm assumptions regarding specific types of statistical techniques being utilized in development of metrics and indicators	Determine potential impact on achieving research and/or program objectives

In the final stage of data verification and validation, exploratory data analysis techniques may be used to identify extreme data points or statistical outliers in the data set. Examples of univariate analysis techniques include the generation and examination of box-and-whisker plots and subsequent statistical tests of any outlying data points. Bivariate techniques include calculation of Spearman correlation coefficients for all pairs of variables in the data set with subsequent examination of bivariate plots of variables having high correlation coefficients. Multivariate techniques have also been used in detecting extreme or outlying values in environmental data sets (Meglen, 1985; Garner et al., 1991; Stapanian et al., 1993). A software package, SCOUT, developed by EPA and based on the approach of Garner et al. (1991) may be used for validation of multivariate data sets.

Suspect data are reviewed to determine the source of error, if possible. If the error is correctable, the data set is edited to incorporate the correct data. If the source of the error cannot be determined, data are qualified as questionable or invalid. Data qualified as questionable may be acceptable for certain types of data analyses and interpretation activities. The decision to use questionable data must be made by the individual data users. Data

qualified as invalid are considered to be unacceptable for use in any analysis or interpretation activities and will generally be removed from the data file and replaced with a missing value code and explanatory comment or flag code. After completion of verification and validation activities, a final data file is created, with copies transmitted for archival and for uploading to the centralized IM system.

Once verified and validated, data files are made available for use in various types of interpretation activities, each of which may require additional restructuring of the data files. These restructuring activities are collectively referred to as "data enhancement." In order to develop indicator metrics from one or more variables, data files may be restructured so as to provide a single record per lake. .

4.2 Data Transfer

Field crews may transmit data electronically via modem or floppy disc; hardcopies of completed data and sample tracking forms may be transmitted to the IM staff at WED via portable facsimile (FAX) machine or via express courier service. Copies of raw, verified, and validated data files are transferred from indicator leads to the IM staff for inclusion in the central IM system. All transfers of data are conducted using a means of transfer, file structure, and file format that has been approved by the IM staff. Data files that do not meet the required specifications will not be incorporated into the centralized data access and management system.

4.3 Hardware and Software Control

All automated data processing (ADP) equipment and software purchased for or used in Lakes Survey surface waters research is subject to the requirements of the federal government, the particular Agency, and the individual facility making the purchase or maintaining the equipment and software. All hardware purchased by EPA is identified with an EPA barcode tag label; an inventory is maintained by the responsible ADP personnel at the facility. Inventories are also maintained of all software licenses; periodic checks are made of all software assigned to a particular PC.

The development and organization of the IM system is compliant with guidelines and standards established by the EMAP Information Management Technical Coordination Group, the EPA Office of Technology, Operations, and Planning (OTOP), and the EPA Office of Administrative Resources Management (OARM). Areas addressed by these policies and guidelines include, but are not limited to, the following:

- Taxonomic Nomenclature and Coding
- Locational data
- Sampling unit identification and reference
- Hardware and software
- Data catalog documentation

The Lakes Survey is committed to compliance with all applicable regulations and guidance concerning hardware and software procurement, maintenance, configuration control, and QA/QC. As new guidance and requirements are issued, the Lakes Survey information

management staff will assess the impact upon the IM system and develop plans for ensuring timely compliance.

4.4 Data Security

All data files in the IM system are protected from corruption by computer viruses, unauthorized access, and hardware and software failures. Guidance and policy documents of EPA and management policies established by the IM Technical Coordination Group for data access and data confidentiality are followed. Raw and verified data files are accessible only to the Lakes Survey collaborators. Validated data files are accessible only to users specifically authorized by the EPA Project Leader. Data files in the central repository used for access and dissemination are marked as read-only to prevent corruption by inadvertent editing, additions, or deletions.

Data generated, processed, and incorporated into the IM system are routinely stored as well as archived on redundant systems. This ensures that if one system is destroyed or incapacitated, IM staff will be able to reconstruct the data bases. Procedures developed to archive the data, monitor the process, and recover the data are described in IM documentation.

Several backup copies of all data files and of the programs used for processing the data are maintained. Backups of the entire system are maintained off-site. System backup procedures are utilized. The central data base is backed up and archived according to procedures already established for WED. All laboratories generating data and developing data files must have established procedures for backing up and archiving computerized data.

4.5 Data Archive

All data will be transferred to U.S. EPA's agency-wide WQX (Water Quality Exchange) data management system for archival purposes. WQX is a repository for water quality, biological, and physical data and is used by state environmental agencies, EPA and other federal agencies, universities, private citizens, and many others. Revised from STORET, WQX provides a centralized system for storage of physical, chemical, and biological data and associated analytical tools for data analysis. Data from the Lakes Survey project in an Excel format will be run through an Interface Module and uploaded to WQX. Once uploaded, states and tribes will be able to download data (using Oracle software) from their region.

5.0 INDICATORS

5.1 Water Chemistry Indicator

5.1.1 Introduction

Trophic indicators based on lake water chemistry information attempt to evaluate lake condition with respect to stressors such as acidic deposition and nutrients as well as other types of physical or chemical contamination. Data are collected for a variety of physical and chemical constituents to provide information on the acid-base status of each lake, water clarity, primary productivity, nutrient status, mass balance budgets of constituents, color, temperature regime, and presence and extent of anaerobic conditions.

There are two components to collecting water chemistry information: collecting samples of lake water for laboratory analysis, and field or *in situ* measurements of dissolved oxygen (DO), pH and water temperature. At each site, crews fill one 4-L cubitainer using a depth-integrated sampler device. All samples are stored in a cooler packed with resealable plastic bags filled with ice and shipped to the analytical laboratory within 24 hours of collection. *In situ* measurements are made using field meters and recorded on standardized data forms. The primary function of the water chemistry information is to determine:

- Acid-base status
- Trophic state (nutrient enrichment)
- Chemical stressors
- Classification of water chemistry type

5.1.2 Field Collection

A single index site is located at the middle of the lake; lake arms are to be ignored when determining the middle of the lake. At the index site, a single 4-L composite sample is collected for laboratory analysis. In addition, a vertical profile of *in situ* or field measurements (temperature, pH and DO) at various depths is conducted to provide a representation of the lake's condition with respect to stratification throughout the water column. The response design for sampling locations is shown in Figure 5-1.

5.1.3 Sampling and Analytical Methods

Sample Collection: At the lake index site, a depth-integrated water chemistry sample is collected from the surface to a depth of 2 m using an integrated sampler device. The entire sample is combined into a single bulk water composite sample. Enough sample should be collected to fill a 4-L cubitainer. Detailed procedures for sample collection and handling are described in the Field Operations Manual.

Field Measurements: At the lake index site, vertical profiles of temperature, pH and DO are measured at predetermined depth intervals. For shallow lakes (<3 m), DO, pH and temperature are measured at the surface and at 0.5-m intervals, until .5 m above the bottom. For lakes deeper than 3.0 m, DO, pH and temperature are measured at the surface and at every meter thereafter through 20 m (or until reaching .5 m above the bottom). After the measurement at 20 m, measurements are recorded every 2 m starting at 22m (or until .5 m above the bottom).

Analysis: A performance-based methods approach is being utilized for water chemistry analysis that defines a set of laboratory method performance requirements for data quality. Following this approach, participating laboratories may choose which analytical methods they will use for each target analyte as long as they are able to achieve the performance requirements as listed in Table 5-1.

5.1.4 Quality Assurance Objectives

Measurement quality objectives (MQOs) are given in Table 5-1. General requirements for comparability and representativeness are addressed in Section 2. The MQOs given in Table 5-1 represent the maximum allowable criteria for statistical control purposes.

For duplicate samples, precision across batches 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 samples of known composition, 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 (see Section 2). Bias (systematic error) is estimated as either net bias or relative net bias (Section 2). Net bias 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 relative bias as the percent difference at the higher concentration range. Precision and bias are monitored at the point of measurement (field or analytical laboratory) by several types of QC samples described in the Section 5.1.6, and from performance evaluation (PE) samples.

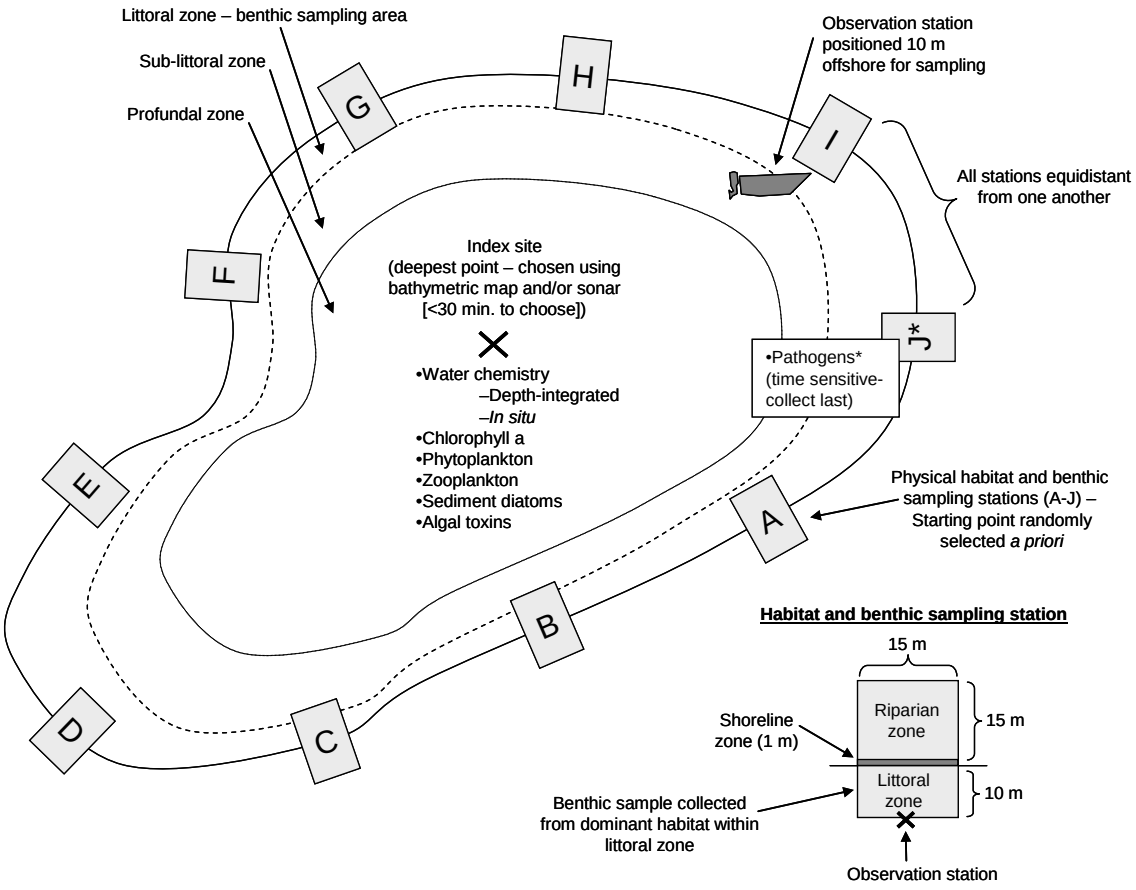

Figure 5-1. Sampling locations for Lakes Survey indicators.

Table 5-1. Performance requirements for water chemistry and chlorophyll-*a* analytical methods.

Analyte	Units	Potential Range of Samples[1]	Long-Term MDL Objective[2]	Laboratory Reporting Limit[3]	Transition Value[4]	Precision Objective[5]	Bias Objective[6]
Conductivity	µS/cm at 25°C	1 to 15,000	NA	2.0	20	± 2 or ±10%	± 2 or 5%
Turbidity	NTU	0 to 44,000	1	2.0	20	± 2 or ±10%	± 2 or ±10%
pH	pH units	3.7 to 10	NA	NA	5.75 and>8.25	± 0.08 or ± 0.15	± 0.05 or ± 0.10
Acid Neutralizing Capacity (ANC)	µeq/L (20 µeq/L=1 mg as $CaCO_3$)	-300 to +75,000 (-16 to 3,750 mg as $CaCO_3$)	NA	NA	±50	± 5 or ±10%	± 5 or ±10%
Total and Dissolved Organic Carbon (TOC/DOC)	mg C/L	0.1 to 109 (as DOC)	0.10	0.20	≤ 1 > 1	± 0.10 or ±10%	± 0.10 or ±10%
Ammonia (NH_3)	mg N/L	0 to 17	0.01 (0.7 µeq/L)	0.02 (1.4 µeq/L)	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Nitrate-Nitrite (NO_3-NO_2)	mg N/L	0 to 360 (as nitrate)	0.01	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Nitrogen (TN)	mg/L	0.1 to 90	0.01	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Phosphorus (TP)	µg P/L	0 to 22,000	2	4	20	± 2 or ±10%	± 2 or ±10%
Ortho-phosphate	µgP/L		2	4	20	± 2 or ±10%	± 2 or ±10%
Sulfate (SO_4)	mg SO_4/L	0 to 5,000	0.25 (5 µeq/L)	0.50 (10 µeq/L)	2.5	± 0.25 or ±10%	± 0.25 or ±10%
Chloride (Cl)	mg Cl/L	0 to 5,000	0.10 (3 µeq/L)	0.20 (6 µeq/L)	1	± 0.10 or ±10%	± 0.10 or ±10%
Nitrate (NO_3)	mg N/L	0 to 360	0.01 (1 µeq/L)	0.02 (4 µeq/L)	0.1	± 0.01 or ±10%	± 0.01 ±10%

Analyte	Units	Potential Range of Samples[1]	Long-Term MDL Objective[2]	Laboratory Reporting Limit[3]	Transition Value[4]	Precision Objective[5]	Bias Objective[6]
Calcium (Ca)	mg Ca/L	0.04 to 5,000	0.05 (2.5 µeq/L)	0.10 (5 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Magnesium (Mg)	mg Mg/L	0.1 to 350	0.05 (4 µeq/L)	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	0.05 (2 µeq/L)	0.10 (4 µeq/L)	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Potassium (K)	mg K/L	0.01 to 120	0.05 (1 µeq/L)	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	0.05	0.10	0.5	± 0.05 or ±10%	± 0.05 or ±10%
Total Suspended Solids (TSS)	mg/L	0 to 27,000	1	2	10	± 1 or ±10%	± 1 or ±10%
True Color	PCU	0 to 350	NA	5	50	±5 or ±10%	±5 or ±10%
Chlorophyll a	µg/L (in extract)	0.7 to 11,000	1.5	3	15	± 1.5 or ±10%	± 1.5 or ±10%

[1] 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.

[2] The long-term method detection limit is determined as a one-sided 99% confidence interval from repeated measurements of a low-level standard across several calibration curves, based on USGS Open File Report 99-193. These represent values that should be achievable by multiple labs analyzing samples over extended periods with comparable (but not necessarily identical) methods.

[3] The minimum reporting limit is the lowest value that need to be quantified (as opposed to just detected), and represents the value of the lowest nonzero calibration standard used. It is set to 2x 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*.

[4] Value at which performance objectives for precision and bias switch from absolute (≤ transition value) to relative 9> 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.

[5] 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.

[6] 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.

5.1.5 Quality Control Procedures: Field Operations

For *in situ* measurements, each field instrument (e.g., multi-probe) must be calibrated, inspected prior to use, and operated according to manufacturer specifications. The measurements will be taken from the surface to the bottom, ending until 0.5 m above the bottom or the maximum depth of 50 m is reached. Figure 5-2 illustrates the general scheme for field chemistry measurement procedures. If problems with any field instrument are encountered, the user should consult the manufacturer's manual, and/or call the manufacturer prior to sampling. In addition to daily calibrations, the DO probe should periodically be checked against a Winkler titration kit to ensure that it is properly calibrated. For pH and conductivity, the calibration of pH electrodes and conductivity probes should be checked using an independent standard that is similar in ionic strength and pH to the lake samples being measured (e.g., Peck and Metcalf 1991, Metcalf and Peck 1993) Specific quality control measures are listed in Table 5-2 for field measurements. Additionally, duplicate samples will be collected at 10% of lakes sampled.

Throughout the water chemistry sample collection process it is important to take precautions to avoid contaminating the sample. Many lakes in some regions have a very low ionic strength (i.e., very low levels of chemical constituents) and samples can be contaminated quite easily by perspiration from hands, sneezing, smoking, suntan lotion, insect repellent, fumes from gasoline engines or chemicals used during sample collection.

5.1.6 Quality Control Procedures: Laboratory Operations

5.1.6.1 Sample Receipt and Processing

QC activities associated with sample receipt and processing are presented in Table 5-3. The communications center and information management staff is notified of sample receipt and any associated problems as soon as possible after samples are received. The general schemes for processing lake water chemistry samples for analysis is presented in Figure 5-3. Several aliquots are prepared from bulk water samples and preserved accordingly. Ideally, all analyses are completed within a few days after processing to allow for review of the results and possible reanalysis of suspect samples within seven days. Critical holding times for the various analyses are the maximum allowable holding times, based on current EPA and American Public Health Association (APHA) requirements (American Public Health Association, 1989).

5.1.6.2 Analysis of Samples

QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. Information regarding QC sample requirements and corrective actions are summarized in Table 5-4. Figure 5-4 illustrates the general scheme for analysis of a batch of water chemistry samples, including associated QC samples.

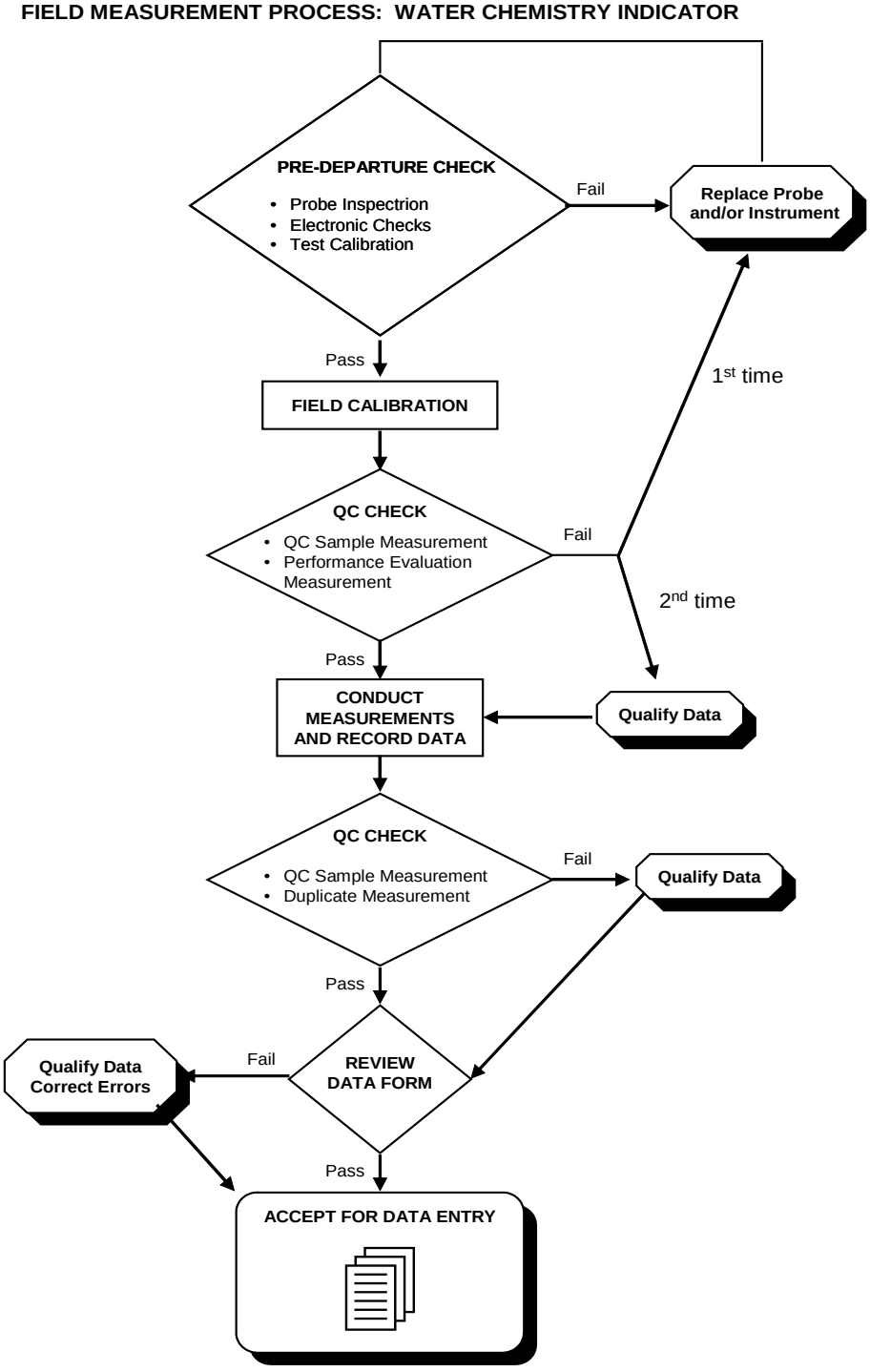

Figure 5-2. Field measurement activities for the water chemistry indicator.

Table 5-2. Field quality control: water chemistry indicator

Check Description	Frequency	Acceptance Criteria	Corrective Actions
Check calibration of instrument	Prior to sampling each day	Specific to each instrument	Adjust and recalibrate, redeploy gear
Verify performance of temperature probe using wet ice.	Prior to initial sampling, daily thereafter	Functionality = ± 2°C	See manufacturer's directions.
Check DO calibration in field against atmospheric standard (ambient air saturated with water)	Weekly	±1.0 mg/L	Adjust and recalibrate
Check calibration of pH, and conductivity with an independent standard solution	Weekly	pH: ≥ 5.75 and ≤ 8.: ± 0.15 < 575 or > 8.25: ± 0.08 Conductivity: ±2 µS/cm or ±10%	Recalibrate or repair/replace electrode or probe

Table 5-3. Sample processing quality control activities: water chemistry indicator

Quality Control Activity	Description and Requirements	Corrective Action
Sample Storage	Store samples in darkness at 4°C Monitor temperature daily	Qualify sample as suspect for all analyses
Holding time	Complete processing bulk samples within 48 hours of collection if possible, or ASAP after receipt	Qualify samples
Aliquot Containers and Preparation	HDPE bottles. Rinse bottles and soak for 48 h with ASTM Type II reagent water; test water for conductivity Prepare bottles to receive acid as preservative by filling with a 10% HCl solution and allow to stand overnight. Rinse six times by filling with deionized water. Determine the conductivity of the final rinse of every tenth bottle. Conductivity must be < 2 µS/cm.	Repeat the deionized water rinsing procedure on all bottles cleaned since the last acceptable check. Check conductivity of final rinse on every fifth bottle.
Filtration	0.4 µm polycarbonate filters required for all dissolved analytes. Rinse filters and filter chamber twice with 50-ml portions of deionized water, followed by a 20-mL portion of sample. Repeat for each filter used on a single sample. Rinse aliquot bottles with two 25 to 50 mL portions of filtered sample before use.	

Table 5-3. Continued.

Quality Control Activity	Description and Requirements	Corrective Action
Preservation	Use ultrapure acids for preservation. Add sufficient acid to adjust to pH < 2. Check pH with indicator paper. Record volume of preservative on container label. Store preserved aliquots in darkness at 4°C until analysis.	
Holding Times for preserved aliquots	Holding times range from 3 days to 6 months, based upon current APHA criteria.	Sample results are qualified as exceeding the specified holding time.

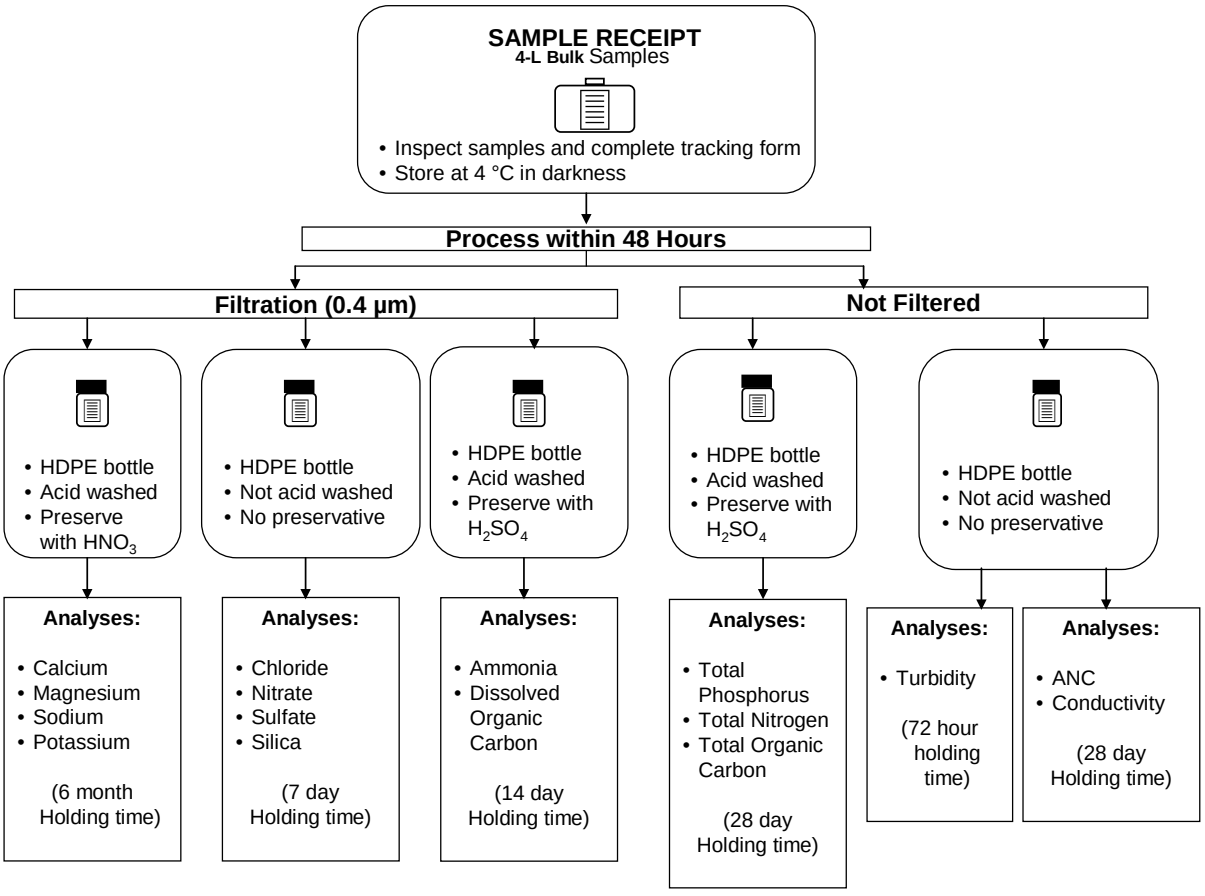

Table 5-4. Laboratory quality control samples: water chemistry indicator

QC Sample Type (Analytes), and Description	Frequency	Acceptance Criteria	Corrective Action
Laboratory/ Reagent Blank: (For all analyses except total suspended solids [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 (U.S. EPA, 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.

Table 5-4. (Continued).

QC Sample Type (Analytes), and Description	Frequency	Acceptance Criteria	Corrective Action
Laboratory Duplicate Sample: (All analyses)	One per batch	Control limits < precision objective	If results are below LRL: Prepare and analyze split from different sample (volume permitting). Review precision of QCCS measurements for batch. Check preparation of split sample. Qualify all samples in batch for possible reanalysis.
Standard Reference Material: (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 which 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.

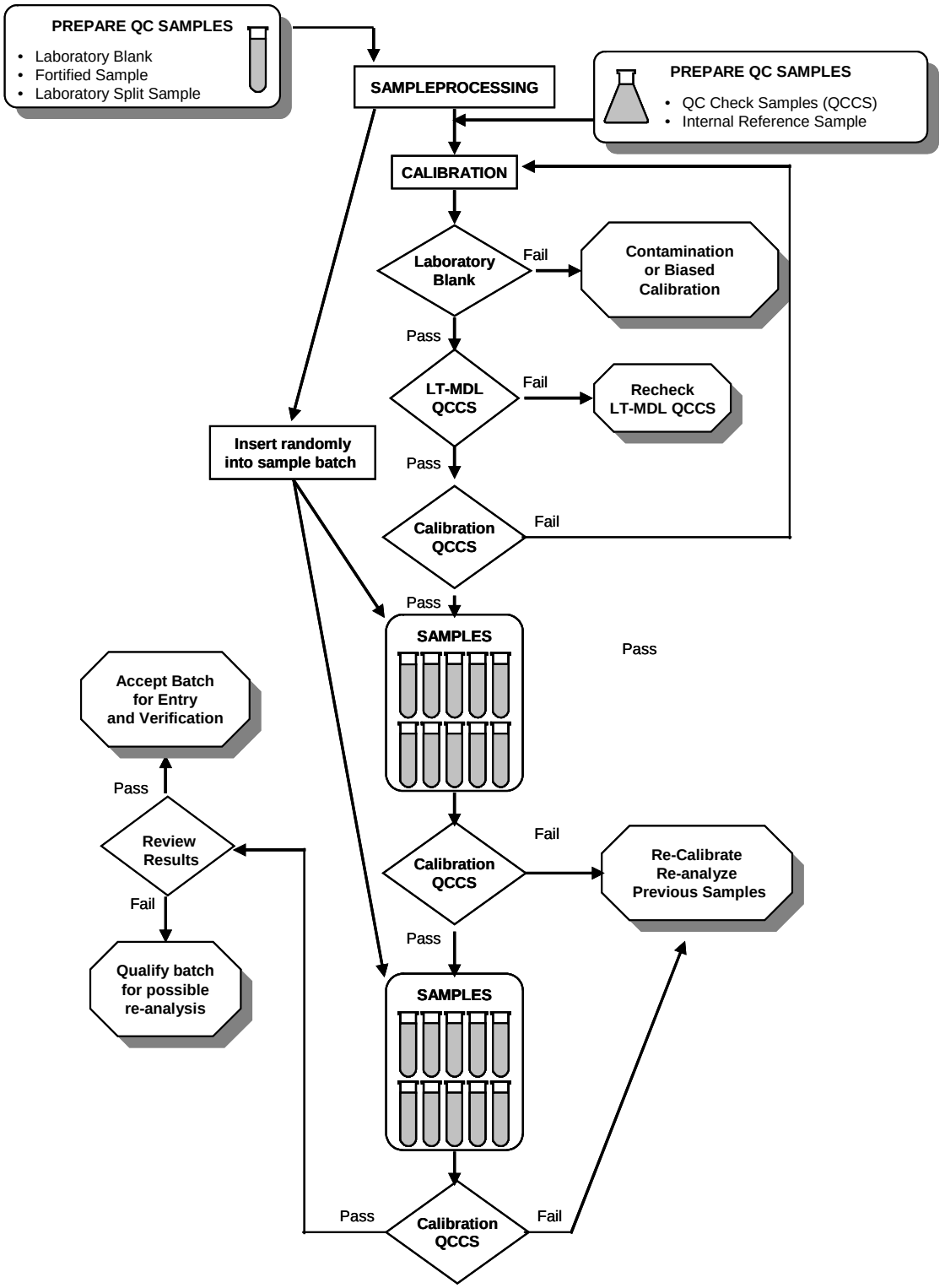

Figure 5-4. Analysis activities for water chemistry samples.

5.1.7 Data Reporting, Review, and Management

Checks made of the data in the process of review and verification are summarized in Table 5-5. Data reporting units and significant figures are given in Table 5-6. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members.

Table 5-5. Data validation quality control: 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 $\leq$100 μeq/L, %IBD $\leq$ $\pm$25%. If total ionic strength > 100 μeq/L, %IBD $\leq\pm$10%. Determine which analytes, if any, are the largest contributors to the ion imbalance. Review suspect analytes for analytical error and reanalyze. If analytical error is not indicated, qualify sample to attribute imbalance to unmeasured ions. Reanalysis is not required. Flag= unacceptable %IBD 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 conductances of major ions in solution (Hillman et al., 1987).	If measured conductivity $\leq$ 25 μS/cm, ([measured – calculated] $\div$ measured) $\leq$ $\pm$25%. If measured conductivity > 25 μS/cm, ([measured – calculated] $\div$ measured) $\leq$ $\pm$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 interlaboratory comparison samples)	Determine impact and possible limitations on overall usability of data

Table 5-6. Data reporting criteria: water chemistry indicator

Measurement	Units	No. Significant Figures	Maximum No. Decimal Places
Dissolved Oxygen	mg/L	2	1
Temperature	°C	2	1
pH	pH units	3	2
Carbon, total & dissolved organic	mg/L	3	1
Acid neutralizing capacity	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
Total suspended solids	mg/L	3	1

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 11

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

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 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 5-7. For the conductivity check, equivalent conductivities for major ions are presented in Table 5-8.

Table 5-7. Constants for converting major ion concentrations from mg/L to □eq/L

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

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

Table 5-8. Factors to calculate equivalent conductivities of major ions[a]

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

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

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

5.2 Chlorophyll-*a* Indicator

5.2.1 Introduction

Trophic indicators based on algal community information attempt to evaluate lake condition with respect to stressors such as nutrient loading. Data are collected for chlorophyll-*a* to provide information on the algal loading and gross biomass of blue-greens and other algae within each lake.

5.2.2 Sampling Design

At the index site located at the deepest point of the lake, a single depth-integrated water sample is collected from the euphotic zone to provide a representation of the lake's trophic condition with respect to its algal loads. The response design for sampling locations is shown in Figure 5-1.

5.2.3 Sampling and Analytical Methods

Sample Collection: At the lake index site, collect a 2-L depth-integrated water sample from the surface within the photic zone (determined for each lake by multiplying the Secchi depth by 2, with a maximum depth of 2 m) using an integrated sampler device. The sample should be preserved immediately on ice and placed in a cooler away from direct light. After returning to shore, the sample is filtered in subdued light to minimize degradation. The filter is then stored in a centrifuge tube on ice before being shipped to the laboratory for chlorophyll-*a* analysis. Detailed procedures for sample collection and processing are described in the Field Operations Manual.

Analysis: A performance-based methods approach is being utilized for chlorophyll-*a* analysis that defines a set of laboratory method performance requirements for data quality. Following this approach, participating laboratories may choose which analytical method they will use to determine chlorophyll-a concentration as long as they are able to achieve the performance requirements as listed in Table 5-1.

5.2.4 Quality Assurance Objectives

MQOs are given in Table 5-1. General requirements for comparability and representativeness are addressed in Section 2. The MQOs given in Table 5-1 represent the maximum allowable criteria for statistical control purposes. LT-MDLs are monitored over time by repeated measurements of low level standards and calculated using Equation 1a.

For precision, the objectives presented in Table 5-1 represent the 99 percent confidence intervals about a single measurement and are thus based on the standard deviation of a set of repeated measurements ($n > 1$). Precision objectives at lower concentrations are equivalent to the corresponding LRL. At higher concentrations, the precision objective is expressed in relative terms, with the 99 percent confidence interval based on the relative standard deviation (Section 2). Objectives for accuracy are equal to the corresponding precision objective, and are based on the mean value of repeated measurements. Accuracy is generally estimated as net bias or relative net bias (Section 2). Precision and bias are monitored at the point of measurement (field or analytical laboratory) by several types of QC samples described in the Section 5.1.6, where applicable, and from performance evaluation (PE) samples.

5.2.5 Quality Control Procedures: Field Operations

Chlorophyll can degrade rapidly when exposed to bright light. It is important to keep the sample on ice and in a dark place (cooler) until it can be filtered. If possible, prepare the sample

in subdued light (or shade) by filtering as quickly as possible to minimize degradation. If the sample filter clogs and the entire sample in the filter chamber cannot be filtered, discard the filter and prepare a new sample, using a smaller volume.

Check the label to ensure that all written information is complete and legible. Place a strip of clear packing tape over the label and bar code, covering the label completely. Record the bar code assigned to the chlorophyll-*a* sample on the Sample Collection Form (Figure 5-5). Also record the volume of sample filtered on the Sample Collection Form. Verify that the volume recorded on the label matches the volume recorded on the Sample Collection Form. Enter a flag code and provide comments on the Sample Collection Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity. Store the filter sample in a 50-mL centrifuge tube (or other suitable container) wrapped in aluminum foil and freeze using dry ice or a portable freezer. Recheck all forms and labels for completeness and legibility. Additionally, duplicate (replicate) samples will be collected at 10% of lakes sampled.

5.2.6 Quality Control Procedures: Laboratory Operations

5.2.6.1 Sample Receipt and Processing

QC activities associated with sample receipt and processing are presented in Table 5-9. The communications center and information management staff are notified of sample receipt and any associated problems as soon as possible after samples are received.

5.2.6.2 Analysis of Samples

QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. Most of the QC procedures described here are detailed in the references for specific methods. However, modifications to the procedures and acceptance criteria described in this QAPP supersede those presented in the methods references. Information regarding QC sample requirements, where applicable, and corrective actions are summarized in Table 5-5.

5.2.7 Data Reporting, Review, and Management

Checks made of the data in the process of review, verification, and validation are summarized in Table 5-10. Data reporting units and significant figures are given in Table 5-11. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members. Once data have passed all acceptance requirements, computerized data files are prepared in a format specified for the Lakes Survey project. The electronic data files are transferred to the Lakes Survey IM Coordinator at WED-Corvallis for entry into a centralized data base. A hard copy output of all files will also be sent to the Lakes Survey IM Coordinator.

SAMPLE COLLECTION FORM-LAKES

LAKE NAME: DATE OF COLLECTION: / / VISIT #: 1 2

LAKE ID: __ __ __ __ __ __ L SITE ID (circle): INDEX OTHER: _____

TEAM ID (circle): 1 2 3 4 5 6 7 8 9 10 OTHER: __

SECCHI DISK TRANSPARENCY

DEPTH DISK DISSAPPEARS	DEPTH DISK REAPPEARS	CLEAR TO BOTTOM (X)	COMMENTS
__ __ . __ __ M	__ __ . __ __ M		

WATER CHEMISTRY (4-L CUBITAINER AND 4 SYRINGES)

SAMPLE ID # (Barcode)	SAMPLE TYPE	DEPTH COLLECTED	FLAG	COMMENTS
_ _ _ _ _ _	R1	M		
_ _ _ _ _ _		M		

CHLOROPHYLL (TARGET VOLUME = 500 mL)

SAMPLE ID # (Barcode)	SAMPLE TYPE	DEPTH COLLECTED	SAMPLE VOLUME	FLAG	COMMENTS
_ _ _ _ _ _	R1	M	mL		
_ _ _ _ _ _		M	mL		

ZOOPLANKTON (FILL TO MARK ON BOTTLE = 80 mL)

MESH SIZE	SAMPLE ID # (Barcode)	SAMPLE TYPE	LENGTH OF TOW	CONTAINERS NO. PRESERVED (✓)		FLAG	COMMENTS
COARSE	_ _ _ _ _ _ _ _	R1	M				
FINE	_ _ _ _ _ _ _ _	R1	M				
	_ _ _ _ _ _ _ _		M				
	_ _ _ _ _ _ _ _		M				

SEDIMENT CORE SAMPLES (TARGET CORE LENGTH = 35 TO 40 CM)

Collected at (circle): INDEX OTHER If OTHER, record direction and distance from INDEX site:

SAMPLE CLASS	SAMPLE ID # (Barcode)	SAMPLE TYPE	LENGTH OF CORE	INTERVAL From	INTERVAL To	FLAG	COMMENTS
TOP	_ _ _ _ _ _ _ _	R1	CM	CM	CM		
BOTTOM	_ _ _ _ _ _ _ _	R1	CM	CM	CM		
	_ _ _ _ _ _ _ _		CM	CM	CM		
	_ _ _ _ _ _ _ _		CM	CM	CM		

FLAG CODES: K = NO MEASUREMENT OR SAMPLE COLLECTED; U = SUSPECT MEASUREMENT OR SAMPLE;
F1. F2, ETC. = MISC. FLAGS ASSIGNED BY EACH FIELD CREW. EXPLAIN ALL FLAGS IN COMMENTS SECTION.

REVIEWED BY (INITIAL):_____

Rev. 3/95 FLDFRMS.95(G) Sample Collection Form - Lakes - 1

Figure 5-5. Sample collection form

Table 5-9. Sample processing quality control: chlorophyll-*a* indicator

Quality Control Activity	Description and Requirements	Corrective Action
Filtration (done in field)	Whatman GF/F (or equivalent) glass fiber filter. Filtration pressure should not exceed 7 psi to avoid rupture of fragile algal cells.	Discard and refilter
Sample Storage	Store samples in darkness and frozen (-20 °C) Monitor temperature daily	Qualify sample as suspect for all analyses

Table 5-10. Data validation quality control: chlorophyll-*a* 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 data from QA samples (e.g., laboratory PE samples or other standards or replicates)	Determine impact and possible limitations on overall usability of data

Table 5-11. Data reporting criteria: chlorophyll-*a* indicator

Measurement	Units	No. Significant Figures	Maximum No. Decimal Places
Chlorophyll-*a*	µg/L	2	1

5.3 Sediment Diatom Indicator

5.3.1 Introduction

Ecological indicators based on sediment diatoms provide an indication of both current and historical lake condition with respect to stressors such as nutrients and sediment loadings. The diatom indicator is unique in that it can potentially provide insight to the "original" or pristine condition of the lake. Diatoms are collected from bottom sediments to provide information on temporal and spatial trends in eutrophication and to provide a historical perspective for comparisons.

5.3.2 Sampling Design

At the index site located at the middle of the lake, a single core is collected from the bottom by lowering a core sampler into the sediment. The collection goals for the diatom sample are to obtain a sample of undisturbed surface sediments, and to obtain a deeper sample

(representing past conditions) that is uncontaminated with the shallower sediments. The response design for sampling locations is shown in Figure 5-1.

5.3.3 Sampling and Analytical Methods

Sample Collection: At the lake index site, a single core is collected from the bottom sediments using a sampling device described by Glew et al. (2001). The target length for a core sample is 35-45 cm in length. If the target length cannot be obtained after two consecutive attempts, the maximum obtainable core should be used. When sampling natural lakes, one sectioned sample is collected from the top 1-cm of the core and another section 1-cm from the bottom. When sampling reservoirs, only the top 1-cm of the core is collected. Each sample is placed in a separate sealable container with a label indicating the depth of the sample and preserved with a wet paper towel to prevent dessication. Detailed procedures for sample collection and handling are described in the Field Operations Manual.

Analysis: Sediment samples are cleaned of organic matter with strong oxidizing agents and slides are made. The analysis is made by identifying and counting 600 individual cells. Detailed procedures for sample processing and enumeration are described in the laboratory methods manual. Table 5-12 summarizes field and analytical methods for the sediment diatom indicator.

Table 5-12. Field and laboratory methods: sediment diatom indicator

Variable or Measurement	QA Class	Expected Range and/or Units	Summary of Method	References
Sample Collection	C	NA	Core sampler used to collect a 35-45 cm core of sediments	Glew et al. 2001; Lakes Survey Field Operations Manual 2006
Sample Digestion and Concentration	N	NA	Add acid and heat at 200°C for 2 hrs. Allow to settle, siphon off supernatant, repeat until final volume is between 25-50 mL	Charles et al. 2003; Lakes Survey Laboratory Methods Manual 2006
Slide preparation	N	NA	Prepare coverslips and mount on slide using Naphrax	Charles et al. 2003; Lakes Survey Laboratory Methods Manual 2006
Enumeration	C	0 to 600 organisms	Random systematic selection of rows and fields with target of 600 organisms from sample	Charles et al. 2003; Lakes Survey Laboratory Methods Manual 2006
Identification	C	genus	Specified keys and references	

C = critical, N = non-critical quality assurance classification.

5.3.4 Quality Assurance Objectives

A taxonomic harmonization table for diatoms will be developed through co-operation of the different taxonomic laboratories to ensure consistent identification among laboratories. The harmonization table will begin with the National Water-Quality Assessment (NAWQA) program diatom list, and taxonomic experts from each laboratory will work together to clean up the data set to ensure that there will be no ambiguous or synonyms taxa in the final data set.

5.3.5 Quality Control Procedures: Field Operations

Any contamination of the samples can produce significant errors in the resulting interpretation. Great care must be taken by the samplers not to contaminate the bottom sample with higher levels of the core or with lake water or with the tools used to collect the sample (i.e., the corer, core tube, and spatulas) and not to mix the surface layer with the deeper sediments. Prior to sampling, the corer device and collection tools should be examined to ensure that they are clean and free of contaminants from previous sampling activities. After the first (top) core is sectioned off, the sectioning apparatus should be removed and rinsed in DI water. This procedure prevents contamination of the bottom sediment layer with diatoms from the upper portion of the core.

After each sample is sectioned and placed in a separate container, the labels should be checked to ensure that the depth of each core is recorded and all written information is complete and legible, and that the label has been completely covered with clear packing tape. It should be verified that the bar code assigned to the sediment diatom sample is recorded correctly on the Sample Collection Form (Figure 5-5). A flag code should be recorded and comments provided on the Sample Collection Form to denote any problems encountered in collecting the sample or the presence of any conditions that may affect sample integrity.

Additionally, duplicate (replicate) samples will be collected at 10% of lakes sampled.

5.3.6 Quality Control Procedures: Laboratory Operations

Specific quality control measures are listed in Table 5-13 for laboratory operations.

Table 5-13. Sample processing quality control: sediment diatom indicator

Quality Control Activity	Description and Requirements	Corrective Action
Sample Storage	Store samples in darkness at 4°C Monitor temperature daily	Qualify sample as suspect for all analyses

5.3.7 Data Reporting, Review, and Management

Checks made of the data in the process of review, verification, and validation are summarized in Table 5-14. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members. Once data have passed all acceptance requirements, computerized data files are prepared in a format specified for the Lakes Survey project. The electronic data files are transferred to the Lakes Survey IM Coordinator at WED-Corvallis for entry into a centralized data base. A hard copy output of all files will also be sent to the Lakes Survey IM Coordinator.

Sample residuals, vials, and slides are archived by each laboratory until the EPA Project Leader has authorized, in writing, the disposition of samples. All raw data (including field data forms and bench data recording sheets) are retained in an organized fashion by the Indicator Team permanently or until written authorization for disposition has been received from the EPA Project Leader.

Table 5-14. 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
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

5.4 Sediment Mercury Indicator

5.4.1 Introduction

Mercury is an important global pollutant of many aquatic ecosystems, and can have a substantial impact to both human and wildlife health. Elevated mercury concentrations, from anthropogenic sources, have increased the levels of mercury in many water bodies through atmospheric deposition. Measuring the extent of sediment mercury in the nation's lakes will attempt to ascertain the potential threat to human and wildlife health. Data are collected to provide information on both total mercury and methyl mercury (MeHg) in the sediment of each lake. Methyl mercury is of interest due to its increased toxicity and bioaccumulation potential, compared to inorganic mercury. Methyl mercury is formed in sediments through microbial conversion of inorganic mercury.

5.4.2 Sample Design

At the index site located at the middle of the lake, a single core is collected from the bottom by lowering a core sampler into the sediment. The collection goals for the mercury sample are to obtain a sample of undisturbed surface sediments. The response design for sampling location is shown in Figure 5-1.

5.4.3 Sampling and Analytical Methods

Sample collection: At the lake index site, a single core is collected from the bottom sediments using a sampling device described by Glew et al. (2001). The sample is taken in conjunction with the sediment diatoms samples (see section 5.3.3). A small amount of sediment, from the top 1-cm section diatom sample, is collected for measuring total and methylmercury. Method for obtaining the top 1-cm section is stated in section 5.3.3. Detailed procedures for sample collection and handling are described in the Field Operations Manual.

Analysis: Mercury sediment samples were analyzed for two different measures of mercury, total and methyl mercury. Laboratories will utilize USGS Techniques and Methods 5A-8 (acid digestion) for sediment total mercury in sediments and USGS Techniques and Methods 5A-7 for sediment methyl mercury. Table 5-15 summarizes field and analytical methods for total and methyl mercury. Performance requirements for both analyte are found in Table 5-16.

5.4.4 Quality Assurance Objectives

Measurement quality objectives (MQOs) are given in Table 5-1. General requirements for comparability and representativeness are addressed in Section 2. The MQOs given in Table 5-17 represent the maximum allowable criteria for statistical control purposes.

For duplicate samples, precision across batches 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 samples of known composition, 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 (see Section 2). Bias (systematic error) is estimated as either net bias or relative net bias (Section 2). Net bias 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 relative bias as the percent difference at the higher concentration range. Precision and bias are monitored at the point of measurement (field or analytical laboratory) by several types of QC samples described in the Section 5.4.6, and from performance evaluation (PE) samples.

5.4.5 Quality Control Procedures: Field Operations

Any contamination of the samples can produce significant errors in the resulting interpretation. Great care must be taken by the samplers not to contaminate the bottom sample with higher levels of the core or with lake water or with the tools used to collect the sample (i.e., the corer, core tube, and spatulas) and not to mix the surface layer with the deeper sediments. Prior to sampling, the corer device and collection tools should be examined to ensure that they are clean and free of contaminants from previous sampling activities. After the first (top) core is sectioned off, the sectioning apparatus should be removed and rinsed in DI water, and once the diatom sampling has been completed, the entire core should be rinsed in DI water. This procedure prevents contamination of the next sample from sediments from the current lake being sampled.

Additionally, duplicate (replicate) samples will be collected at 10% of lakes sampled.

Table 5-15. Field and laboratory methods: total mercury and methyl mercury

Variable or Measurement	QA Class	Expected Range and/or Units	Summary of Method	References
Sample Collection	C	NA	Core sampler used to collect a 35-45 cm core of sediments	Glew et al. 2001; Lakes Survey Field Operations Manual 2006

Table 5-15. Continued

Sample Digestion, Preparation, and Analysis	C	NA	Total mercury: an aliquot of solid material is homogenized with a Teflon policemen digestion bomb with aqua regia at room temperature overnight to convertall Hg to Hg^{2+}. Sample is then diuted to volume with 5% BrCl. Sample is then pre-reduced with NH2OH*HCl) to remove free halogens, then reduced again to remove Hg^{2+} to Hg^{0}. The sample is then analyzed using a cold vapor atomic fluorescence spectrometer. Methyl mercury: solids are placed in a centrifuge tube, then KBr, $CuSO_4$, and CH_2Cl_2 are sequentially added. Mixture is allowed to react for an hour. An aliquot of CH_2Cl_2 is cleanly transferred to a vial of reagent water. The vial is heated until the CH_2Cl_2 has been evaporated and MeHg has been backextracted into the reagent water. The extractant is then ethylated using $NaBEt_4$ and allowed to react for 15 minutes. The sample is then purged with nitrogen gas and the ethylated Hg is then collected in a Carbotrap. The samples are then run through a gas chromatographic column, and then detected using cold vapor atomic fluorescence spectrometry.	Olund et al. 2005; Dewild et al. 2005

Table 5-16. Performance requirements for total mercury and methyl mercury

Analyte	Units	Laboratory Reporting Limit[3]	Precision Objective[5]	Bias Objective[6]
Total Mercury	ng/analytical aliquot	0.3	±10%	±10%
Methyl Mercury	ng/g	0.08	±10%	±10%

5.4.6 Quality Control Procedures: Laboratory Operations

5.4.6.1 Sample Receipt and Processing

QC activities associated with sample receipt and processing are presented in Table 5-17. The communications center and information management staff is notified of sample receipt and any associated problems as soon as possible after samples are received. Several sediment samples are prepared from sediment core samples and preserved accordingly. Ideally, all analyses are completed within a few days after processing to allow for review of the results.

5.4.6.2 Analysis of Samples

QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. Information regarding QC sample requirements and corrective actions are summarized in USGS Techniques and Methods 5A-8 (total mercury) and 5A-7 (methyl mercury) (Olund et al. 2005, Dewild et al. 2005).

5.4.7 Data Reporting, Review, and Management

Checks made of the data in the process of review and verification are summarized in Table 5-18. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members.

5.5 Physical Habitat Quality Indicator

5.5.1 Introduction

The physical habitat shoreline and littoral surveys that the Lakes Survey field teams conduct serve three purposes. First, this habitat information is absolutely essential to the interpretation of what lake biological assemblages "should" be like in the absence of many types of anthropogenic impacts. Second, the habitat evaluation is a reproducible, quantified estimate of habitat condition, serving as a benchmark against which to compare future habitat changes that might result from anthropogenic activities. Third, the specific selections of habitat information collected aid in the diagnosis of probable causes of ecological impairment in lakes.

In addition to information collected in the field by the shoreline and littoral surveys, the physical habitat description of each lake includes many map-derived variables such as lake surface area, shoreline length, and shoreline complexity. Furthermore, an array of information, including watershed topography and land use, supplements the physical habitat information. The
C = critical, N = non-critical quality assurance classification.

Table 5-17. Sample processing quality control activities: total and methyl mercury

Quality Control Activity	Description and Requirements	Corrective Action
Sample Storage	Store samples at -15°C Monitor temperature daily	Qualify sample as suspect for all analyses
Aliquot Containers and Preparation	Teflon vials (cleaned according to DeWild et al. (2002), baked glass vials (prepared by heating to 550°C for 4 hours), or acid-rinsed polycarbonate vials.	
Holding Times for frozen samples	There are currently no studies on holding times 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.	

Table 5-18. Data validation quality control: total and methyl mercury

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 data from QA samples (laboratory PE samples, and interlaboratory comparison samples)	Determine impact and possible limitations on overall usability of data

shoreline and littoral surveys concentrate on information best derived "on the ground." As such, these survey results provide the all-important linkage between large watershed-scale influences and those forces that directly affect aquatic organisms day to day. Together with water chemistry, the habitat measurements and observations describe the variety of physical and chemical conditions that are necessary to support biological diversity and foster long-term ecosystem stability. These characteristics of lakes and their shorelines are the very aspects that are often changed as a result of anthropogenic activities.

5.5.2 Sampling Design

As the physical habitat indicator is based on field measurements and observations, there is no sample collection associated with this indicator. The shoreline and littoral habitat surveys employ a randomized, systematic design with 10 equally spaced observation stations located around the shore of each sample lake. Teams go to the field with pre-marked lake outlines showing these stations. The response design for sampling locations is shown in Figure 5-1.

5.5.3 Sampling Methods

Field Measurements: Field measurements, observations, and associated methodology for the protocol are summarized in Table 5-19. The observations at each station include quantitative and semiquantitative observations of vegetation structure, anthropogenic disturbances, and bank substrate onshore. In-lake littoral measurements and observations deal with littoral water depth, bottom substrate, nearshore fish cover, and aquatic macrophyte cover. With quantifiable confidence, investigators condense these observations into descriptions applicable to the whole lakeshore and littoral zone. Detailed procedures for completing the protocol are provided in the Field Operations Manual; equipment and supplies required are also listed. All measurements and observations are recorded on standardized forms which are later entered in to the central EMAP surface waters information management system (SWIM) at WED-Corvallis.

There is no sample collection or laboratory analysis associated with the physical habitat measurements.

Table 5-19. Field measurement methods: physical habitat indicator.

Variable or Measurement	Units		Summary of Method	
RIPARIAN ZONE				
Vegetation type	None		Record the dominant vegetation type in the canopy and understory layers within plot	
Riparian vegetation structure	percent		Visually estimate areal coverage of ground cover, understory, and canopy types within plot	
Substrate type	percent		Visually estimate areal coverage of substrate types present in area 1 m back from water	
Bank angle	none		Describe the angle of the shoreline bank back 1 m from the edge of the water	
Bank features	0.1 m		Visually estimate the vertical and horizontal distances between the present lake level and the high water line	
Human influence	none		Estimate presence/absence of defined types of anthropogenic features	
LITTORAL ZONE				
Substrate type	percent		Visually estimate areal coverage of substrate types present within the 10-by 15-m area between the boat and shoreline	

Table 5-19. Continued

Station depth	m		Measure depth at 10 m offshore	
Surface film	none		Indicate presence/absence of defined types of surface films	
Sediment color	none		Note sediment color if a sample can be seen or collected	
Sediment odor	none		Note sediment odor if a sample can be collected	
Macrophyte cover	percent		Estimate areal coverage of aquatic macrophyte types: submerged, emergent, and floating within the 10-by 15-m area between the boat and shoreline	
	none		Indicate presence/absence of fish cover types within the 10-by 15-m area between the boat and shoreline	
Littoral habitat and cover	none		Classify littoral habitat according to the following: disturbance regime, cover class, cover type, and substrate type for 10 m by 15 m littoral area	

5.5.4 Quality Assurance Objectives

MQOs are given in Table 5-20. General requirements for comparability and representativeness are addressed in Section 2. The MQOs given in Table 5-20 represent the maximum allowable criteria for statistical control purposes. Precision is determined from results of revisits (field measurements) taken on a different day and by duplicate measurements taken on the same day.

5.5.5 Quality Control Procedures: Field Operations

Specific quality control measures are listed in Table 5-21 for field measurements and observations.

Table 5-20. Field quality control: physical habitat indicator

Check Description	Frequency	Acceptance Criteria	Corrective Actions
QUALITY CONTROL			
Check totals for cover class categories (vegetation type,	Each station	Sum must be reasonable	Repeat observations

substrate, cover)			
Check completeness of station depth measurements	Each station	Depth measurements for all stations	Obtain best estimate of depth where actual measurement not possible
DATA VALIDATION			
Estimate precision of measurements based on repeat visits	2 visits	Measurements should be within 10 percent	Review data for reasonableness; Determine if acceptance criteria need to be modified

5.5.6 Quality Control Procedures: Laboratory Operations

There are no laboratory operations associated with this indicator.

Table 5-21. Measurement data quality objectives: physical habitat indicator

Variable or Measurement	Precision	Accuracy	Completeness
Field Measurements and Observations	±10%	NA	90%

NA = not applicable in most cases. This would apply if the field auditor did a separate assessment and compared the results to the crews.

5.5.7 Data Management, Review, and Validation

Checks made of the data in the process of review, verification, and validation are summarized in Table 5-22. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members. All raw data (including all standardized forms and logbooks) are retained permanently in an organized fashion in accordance with EPA records management polices.

5.6 Phytoplankton Indicator

5.6.1 Introduction

Phytoplankton are free-floating algae suspended in the lakes' water column, which provide the base of most lake food webs. Excessive nutrient and organic inputs from human activities in lakes and their watersheds lead to eutrophication, characterized in part by increases in phytoplankton biomass. Both species composition and abundance respond to water quality changes caused by nutrients, pH, alkalinity, temperature, and metals.

5.6.2 Sampling Design

At the index site located at the middle of the lake, a single depth-integrated phytoplankton sample is collected from the euphotic zone of sufficient volume to ensure adequate phytoplankton biomass for analysis. The response design for sampling locations is shown in Figure 5-1.

5.6.3 Sampling and Analytical Methods

Sample Collection: An integrated sampling device is used to collect a depth-integrated water sample from the euphotic zone. Sample depth was determined by depth of the euphotic zone (2 times the secchi depth). However, if the Secchi depth is less than 1 m, the sampler should be held at an oblique angle down to a depth of twice the Secchi depth. This is to ensure that the sample is collected from the upper epilimnion. From the 2-L composite sample collected, an aliquot of 1-L is transferred to a bottle for settling and preserved with Lugol's solution. The remaining 1-L aliquot is used for the algal toxin sample (see section 5.8). Detailed procedures for sample collection and handling are described in the Field Operations Manual.

Analysis: Preserved samples are processed, enumerated, and organisms identified to the lowest possible taxonomic level (generally genus, see Laboratory Methods Manual) using specified standard keys and references. Processing and archival methods are based on USGS NAWQA methods (Charles et al. 2003). Detailed procedures are contained in the laboratory operations manual and cited references. There is no maximum holding time associated with preserved phytoplankton samples. Table 5-22 summarizes field and analytical methods for the phytoplankton indicator.

5.6.4 Quality Assurance Objectives

To ensure valid taxonomic data for phytoplankton, laboratories will reanalyze 10% of all taxonomic samples. This procedure will include re-counts of soft algae subsamples and diatom slides, evaluation of taxonomic accuracy and a complete re-processing and re-count of selected quality control samples. Additionally, quantitative comparisons among counts will be assessed using Jaccard's Index and percent similarity. Once bench taxonomist are finished with this process a minimum of 10% of all samples will be re-analyzed by an independent phycologist to ensure taxonomic accuracy and reproducibility of the processing and analysis methods.

Table 5-22. Field and laboratory methods: phytoplankton indicator

Variable or Measurement	QA Class	Expected Range and/or Units	Summary of Method	References
Sample Collection	C	NA	Depth-integrated sampler used to collect 1-L water sample from euphotic zone	Lakes Survey Field Operations Manual 2006

Table 5-22. Continued

Concentrate Subsamples	N	NA	Concentrated by settling and decanting or by centrifugation to 5-10 times the original whole-water sample	Charles et al.; 2003 Lakes Survey Laboratory Methods Manual 2006
Counting cell/ Chamber preparation	N	NA	Prepare either Palmer-Maloney counting cell or Utermöhl sedimentation chamber	Charles et al.; 2003 Lakes Survey Laboratory Methods Manual 2006
Enumeration	C	0 to 300 organisms	Random systematic selection of field or transect with target of 300 organisms from sample	Charles et al. 2003; Lakes Survey Laboratory Methods Manual 2006
Identification	C	genus	Specified keys and references	

C = critical, N = non-critical quality assurance classification.

5.6.5 Quality Control Procedures: Field Operations

After the 1-L bottle has been filled and Lugol's preservative has been added, the label should be checked to ensure that all written information is complete and legible, and that the label has been completely covered with clear packing tape. It should be verified that the bar code assigned to the phytoplankton sample is recorded correctly on the Sample Collection Form (Figure 5-5). The presence of preservative in the sample should be noted on the Sample Collection Form to assure the integrity of the sample. A flag code should be recorded and comments provided on the Sample Collection Form to denote any problems encountered in collecting the sample or the presence of any conditions that may affect sample integrity.

Additionally, duplicate (repeat) samples will be collected at 10% of lakes sampled.

5.6.6 Quality Control Procedures: Laboratory Operations

It is critical that prior to taking a small portion of the subsample, the sample be thoroughly mixed and macro or visible forms are evenly dispersed. Specific quality control measures are listed in Table 5-24 for laboratory identification operations.

5.6.7 Data Management, Review, and Validation

Checks made of the data in the process of review, verification, and validation are summarized in Table 5-23. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members. Once data have passed all acceptance requirements, computerized data files are prepared in a format specified for the Lakes Survey project. The electronic data files are transferred to the Lakes Survey IM Coordinator at WED-Corvallis for entry into a centralized data base. A hard copy output of all files will also be sent to the Lakes Survey IM Coordinator.

Sample residuals, vials, and slides are archived by each laboratory until the EPA Project Leader has authorized, in writing, the disposition of samples. All raw data (including field data forms and bench data recording sheets) are retained permanently in an organized fashion by the Indicator Lead in accordance with EPA records management policies.

Table 5-23. 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 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
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

5.7 Zooplankton Indicator

5.7.1 Introduction

Zooplankton are important components of the open water environment of lakes and ponds. Most species are microscopic and consist of crustaceans, rotifers, pelagic insect larvae, and aquatic mites. Zooplankton are important elements of the food chain since they transfer energy from algae (primary producers) to larger invertebrate predators and fish. The zooplankton species assemblage responds to environmental stressors such as nutrient enrichment, acidification, and fish stocks. The effects of environmental stress can be detected through changes in species composition and abundance, body size distribution, and food web structure.

5.7.2 Sampling Design

At the index site located at the deepest point of the lake, a single zooplankton sample is collected to provide a representation of the lake's condition with respect to its biota. The response design for sampling locations is shown in Figure 5-1.

5.7.3 Sampling and Analytical Methods

Sample Collection: Zooplankton samples are collected using a Wisconsin net sampler with one fine (80 µm) and one coarse (243 µm) mesh net towed vertically from near the bottom to the surface. A calibrated chain is used to make and measure the vertical tow. The chain is attached to the Wisconsin net so that depth is measured from the mouth of the net. The net is hauled from about 0.5 m off the bottom to the surface. In clear, shallow lakes (less than 2-m deep, where the Secchi disk can be seen on the bottom), a second tow is performed to collect a sufficient number of individuals to adequately characterize the assemblage. Detailed procedures for sample collection and handling are described in the Field Operations Manual.

Analysis: Preserved samples are processed, enumerated, and organisms identified to the lowest possible taxonomic level (generally genus, see Laboratory Methods Manual) using specified standard keys and references. Processing and archival methods are based on standard methods. Detailed procedures are contained in the Laboratory Methods Manual and cited references. There is no maximum holding time associated with preserved zooplankton samples. Table 5-24 summarizes field and analytical methods for the zooplankton indicator.

5.7.4 Quality Assurance Objectives

To ensure valid taxonomic data for zooplankton, laboratories will reanalyze 10% of all taxonomic samples. This procedure will include re-counts of subsamples, evaluation of taxonomic accuracy and a complete re-processing and re-count of selected quality control samples. Once bench taxonomist are finished with this process a minimum of 10% of all samples will be re-analyzed by an independent zooplankton taxonomist to ensure taxonomic accuracy and reproducibility of the processing and analysis methods.

Table 5-24. Field and laboratory methods: zooplankton indicator

Variable or Measurement	QA Class	Expected Range and/or Units	Summary of Method	References
Sample Collection	C	NA	Wisconsin nets with 80 µm and 243 µm mesh towed vertically from 0.5m above bottom to surface	Lakes Survey Field Operations Manual 2006
Subsampling	N	NA	Pipette from graduated cylinder/ Imhoff Cone or Folsom plankton splitter	Lakes Survey Laboratory Methods Manual 2006
Counting cell/ Chamber preparation	N	NA	Prepare counting cell for small organisms and counting chamber for larger organisms	Lakes Survey Laboratory Methods Manual 2006
Enumeration	C	400 organisms	Random systematic selection of field with target of 400 organisms from sample	Lakes Survey Laboratory Methods Manual 2006
Identification	C	genus	Specified keys and references	

C = critical, N = non-critical quality assurance classification.

5.7.5 Quality Control Procedures: Field Operations

After the sample is collected and dispensed into 125 mL jars, the labels should be checked to verify that all written information is complete and legible, and that the label has been completely covered with clear packing tape. It should be verified that both the bar codes assigned to the sample and the tow length have been recorded correctly on the Sample Collection Form (Figure 5-5). The presence of preservative in the sample should be noted on the Sample Collection Form to assure the integrity of the sample. A flag code should be recorded and comments provided on the Sample Collection Form to denote any problems encountered in collecting the sample or the presence of any conditions that may affect sample integrity.

Additionally, duplicate (repeat) samples will be collected at 10% of lakes sampled.

5.7.6 Quality Control Procedures: Laboratory Operations

Specific quality control measures are listed in Table 5-24 for laboratory operations.

5.7.7 Data Management, Review, and Validation

Checks made of the data in the process of review, verification, and validation are summarized in Table 5-25. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members. Once data have passed all acceptance requirements, computerized data files are prepared in a format specified for the Lakes Survey project. The electronic data files are transferred to the Lakes Survey IM Coordinator at WED-Corvallis for entry into a centralized data base. A hard copy output of all files will also be sent to the Lakes Survey IM Coordinator.

Sample residuals, and vials are archived by each laboratory until the EPA Project Leader has authorized, in writing, the disposition of samples. All raw data (including field data forms and bench data recording sheets) are retained permanently in an organized fashion by the Indicator Lead in accordance with EPA records management policies.

Table 5-25. 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

Table 5-25. Continued

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
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

5.8 Pathogen Indicator

5.8.1 Introduction

The primary function of collecting water samples for Pathogen Indicator Testing is to provide a relative comparison of fecal pollution indicators for national lakes and ponds. The concentration of *Enterococci* (the current bacterial indicator for fresh and marine waters) in a water body correlates with the level of more infectious gastrointestinal pathogens present in the water body. While some Enterococci are opportunistic pathogens among immuno-compromised human individuals, the presence of Enterococci is more importantly an indicator of the presence of more pathogenic microbes (bacteria, viruses and protozoa) associated with human or animal fecal waste. These pathogens can cause waterborne illness in bathers and other recreational users through exposure or accidental ingestion. Disease outbreaks can occur in and around beaches that become contaminated with high levels of pathogens. Therefore, measuring the concentration of pathogens present in lake and pond water can help assess comparative human health concerns regarding recreational use.

In this survey, a novel, Draft EPA Quantitative PCR Method (1606) will be used to measure the concentration of genomic DNA from the fecal indicator group *Enterococcus* in the water samples. While neither federal or state Water Quality Criteria (standards) have been formally established for the level of *Enterococcus* DNA in a sample, epidemiological studies (Wade *et al.* 2005) have established a strong correlation between *Enterococcus* DNA levels and the incidence of high-credible gastrointestinal illness (HCGI) among swimmers. The *Enterococcus* qPCR results will serve as an estimate of the concentration of total (culturable and non-culturable) Enterococci present in the surveyed lakes and ponds for the purpose of comparative assessment. This study also has the potential to yield invaluable information about the inhibitory effects of water matrices from the different regions of the nation upon the qPCR assay.

5.8.2 Sampling Design

A single "pathogen" water sample will be collected from one sampling location approximately 10 m offshore, in conjunction with the final physical habitat sampling station location. The plot design for sampling locations is shown in Figure 5-1.

5.8.3 Sampling and Analytical Methods

Sample Collection: At the final physical habitat shoreline station (located approximately 10 m off shore), a single 250-mL water grab sample is collected approximately 6-12 inches below the surface of the water. Sodium thiosulfate tablets are added to the sample to de-chlorinate the water. Detailed procedures for sample collection and handling are described in the Field Operations Manual. Pathogen samples must be filtered and the filters must be folded and frozen in vials within 6 hours of collection.

Analysis: Pathogen samples are filter concentrated, then shipped on dry ice to the New England Regional Laboratory where the filter retentates are processed, and the DNA extracts are analyzed using Quantitative Polymerase Chain Reaction (QPCR), a genetic method that quantifies a DNA target via a fluorescently tagged probe, based on methods developed by USEPA National Exposure Research Laboratory. Detailed procedures are contained in the laboratory operations manual. Table 5-26 summarizes field and analytical methods for the pathogen indicator.

5.8.4 Quality Assurance Objectives

Measurement quality objectives (MQO) are given in table 5-27. General requirements for comparability and representativeness are addressed in Section 2. Precision is calculated as percent efficiency, estimated from independent identifications of organisms in randomly selected samples. The MQO for accuracy is evaluated by having individual specimens representative of selected taxa identified by recognized experts.

5.8.5 Quality Control Procedures: Field Operations

It is important that the sample container be completely sterilized and remain unopened until samples are ready to be collected. Once the sample bottles are lowered to the desired depth (6-12 in. below the surface), the sample bottles may then be opened and filled. After filling the 250-mL bottle, discard a small portion of the sample and add the sodium thiosulfate tablet to the sample for de-chlorination. Check the label to ensure that all written information is complete and legible. Place a strip of clear packing tape over the label and bar code, covering the label completely. Record the bar code assigned to the pathogen sample on the Sample Collection Form (Figure 5-5). Enter a flag code and provide comments on the Sample Collection Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity. All samples should be placed in coolers and maintained on ice during transport to the laboratory and maintained at 1–4°C during the time interval before they are filtered for analysis. Recheck all forms and labels for completeness and legibility.

Field blanks and duplicates will be collected at 10% of sites sampled. In addition, each field crew should collect a blank sample over the course of the survey as a check on each crew's aseptic technique and the sterility of test reagents and supplies.

Table 5-26. Field and laboratory methods: pathogen indicator (Enterococci)

Variable or Measurement	QA Class	Expected Range and/or Units	Summary of Method	References
Sample Collection	C	NA	Sterile sample bottle submerged to collect 250-mL sample 6-12" below surface at 10m from shore	Lakes Survey Field Operations Manual 2006
Sub-sampling	N	NA	2 x 50-mL sub-samples poured in sterile 50-mL tube after mixing by inversion 25 times.	Lakes Survey Laboratory Methods Manual 2006
Sub-sample (& Buffer Blank) Filtration	N	NA	Up to 50-mL sub-sample filtered through sterile polycarbonate filter. Funnel rinsed with minimal amount of buffer. Filter folded, inserted in tube then frozen.	Lakes Survey Laboratory Methods Manual 2006
Preservation & Shipment	C	-40Cto+40 C	Batches of sample tubes shipped on dry ice to lab for analysis.	Lakes Survey Laboratory Methods Manual 2006
DNA Extraction (Recovery)	C	10-141%	Bead-beating of filter in buffer containing Extraction Control (SPC) DNA. DNA recovery measured	EPA Draft Method 1606 *Enterococcus* qPCR
Method 1606 (*Enterococcus* & SPC qPCR)	C	<60 (RL) to >100,000 ENT CCEs /100-mL	5-uL aliquots of sample extract are analyzed by ENT & Sketa qPCR assays along with blanks, calibrator samples & standards. Field and lab duplicates are analyzed at 10% frequency. Field blanks analyzed at end of testing only if significant detections observed.	EPA Draft Method 1606 *Enterococcus* qPCR NERL NLPS2007 qPCR Analytical SOP

C = critical, N = non-critical quality assurance classification.

Table 5-27. Measurement data quality objectives: pathogen-indicator DNA sequences

Variable or Measurement*	Method Precision	Method Accuracy	Completeness
SPC & ENT DNA sequence numbers of Calibrators & Standards by AQM	RSD=50%	50%	95%
ENT CCEs by dC*t* RQM	RSD = 70%	35%	95%
ENT CCEs by ddC*t* RQM	RSD = 70%	50%	95%

*AQM = Absolute Quantitation Method; RQM = Relative Quantitation Method;
SPC = Sample Processing Control (Salmon DNA / Sketa); CCEs = Calibrator Cell Equivalents

5.8.6 Quality Control Procedures: Laboratory Operations

Specific quality control measures are listed in Table 5-29 for laboratory operations

Table 5-28. Laboratory quality control: pathogen-indicator DNA sequences

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
SAMPLE PROCESSING			
Re-process sub-samples (Lab Duplicates)	10% of all samples completed per laboratory	Percent Congruence <70% RSD	If >70%, re-process additional sub-samples
qPCR ANALYSIS			
Duplicate analysis by different biologist within lab	10% of all samples completed per laboratory	Percent Congruence ≤70% RSD	If >70%, determine reason and if cause is systemic, re-analyze all samples in question.
Independent analysis by external laboratory	None	Independent analysis TBD	Determine if independent analysis can be funded and conducted.
Use single stock of *E. faecalis* calibrator	For all qPCR calibrator samples for quantitation	All calibrator sample C*p* (C*t*) must have an RSD ≤ 50%.	If calibrator C*p* (C*t*) values exceed an RSD value of 50% a batch's calibrator samples shall be re-analyzed and replaced with new calibrators to be processed and analyzed if RSD not back within range.
DATA PROCESSING & REVIEW			
100% verification and review of qPCR data	All qPCR amplification traces, raw and processed data sheets	All final data will be checked against raw data, exported data, and calculated data printouts before entry into LIMS and upload to Corvallis, OR database.	Second tier review by contractor and third tier review by EPA.

5.8.7 Data Management, Review, and Validation

Once data have passed all acceptance requirements, computerized data files are prepared in a format specified by the 2007 NLPS. The electronic data files are transferred to the Lakes Survey IM Coordinator at WED-Corvallis for entry into a centralized data base. A hard copy output of all files will also be sent to the Lakes Survey IM Coordinator.

5.9 Algal Toxin Indicator

5.9.1 Introduction

Cyanobacteria, also known as blue-green algae, are photosynthetic bacteria found in eutrophic waters. Hundreds of bioactive compounds have been isolated from cyanobacteria including numerous cyanotoxins, which have been known to threaten human health due to contaminated drinking water and consumption of contaminated aquatic organisms. The most common of the toxin groups produced and released by cyanobacteria are microcystins. Measuring the concentration of microcystins in the water provides and indication of the safety of the lake water for recreational purposes.

5.9.2 Sampling Design

At the index site located at the deepest point of the lake, a single depth-integrated water sample is collected from the euphotic zone to provide an indication of the presence and concentration of potentially hazardous algal toxins. The response design for sampling locations is shown in Figure 5-1.

5.9.3 Sampling and Analytical Methods

Sample Collection: An integrated sampling device is used to collect a depth-integrated water sample from the surface down to a depth of 2 m. However, if the Secchi depth is less than 1 m, the sampler should be held at an oblique angle down to a depth of twice the Secchi depth. From the 2-L composite sample collected, an aliquot of 400 mL should be collected in a pre-rinsed 500 mL HDPE or amber glass bottle and placed immediately in a cooler with ice, or frozen using dry ice if possible. More detailed procedures for sample collection and handling are described in the Field Operations Manual.

Analysis: A performance-based approach is being utilized for the Mirocystin analysis that defines a set of laboratory methods performance requirements for data quality. Preserved samples are processed and concentrations reported using a microtiter plate Enzyme-Linked Immuno-Sorbent Assay (ELISA) using the Abraxis kit. Laboratory work will be performed by the UGSG Organic Geochemistry Research Group (OGRG) Laboratory in Lawrence, Kansas.

5.9.4 Quality Assurance Objectives

Results from water samples and concentrations are reported between 0.10 µg/L and 5.0 µg/L without dilution. If a dilution is performed, higher concentrations can be reported. Non-detected are reported as "<0.10 µg/L".

5.9.5 Quality Control Procedures: Field Operations

It is important that the sample bottle be rinsed with sample water three times before collecting the sample. After collecting 400 mL of sample water and sealing the lid with electrical tape, check the label to ensure that all written information is complete and legible. Place a strip of clear packing tape over the label and bar code, covering the label completely. Record the bar code assigned to the algal toxin sample on the Sample Collection Form (Figure 5-5). Enter a flag code and provide comments on the Sample Collection Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity. All samples should be placed in coolers and maintained on ice during transport to the laboratory and frozen immediately upon return to the lab. Recheck all forms and labels for completeness and legibility. Additionally, field duplicate and field replicate samples will be collected at 10% of lakes sampled.

5.9.6 Quality Control Procedures: Laboratory Operations

5.9.6.1 Sample Receipt and Processing

The communications center and information management staff is notified of sample receipt and any associated problems as soon as possible after samples are received.

5.9.6.2 Analysis of Samples

QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. Information regarding QC sample requirements and corrective actions are summarized in Table 5-29.

Samples will be analysis by the USGS using the a microtiter plate Enzyme-Linked Immuno-Sorbent Assay (ELISA) using the Abraxis kit. An example layout of this kit is shown in Table 5-30. The SoftMax Pro software is on the immunoassay computer and is used for controlling the microtiter plate reader and for calculating results. The 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. The lower reporting limit is 0.10 µg/L. Samples with less than this concentration are flagged as non-detects.

Laboratory duplicates should have a percent Relative Standard Deviation (%RSD) of <20 percent when compared to each other. Laboratory Spiked Duplicates must have an actual value

of +/-20 percent of the theoretical concentration of the spiked sample. The theoretical concentration is determined by adding the concentration of the unspiked sample and .75 µg/L

For each set of ten samples, the first and 5th samples are duplicates, and the tenth sample is a spiked duplicate. A designated archived project samples is re-analyzed with every set that is run. Control charts are maintained for these samples. A running historical average is maintained

5.9.7 Data Management, Review, and Validation

Once data have passed all acceptance requirements, computerized data files are prepared in a format specified for the Lakes Survey project. An example data entry sheet is shown below in Figure 5-6. The electronic data files are transferred to the Lakes Survey IM Coordinator (Marlys Cappaert) for entry into a centralized data base. A hard copy output of all files will also be sent to the Lakes Survey IM Coordinator.

Table 5-29. 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	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

Table 5-30. Example layout of samples and controls on microtiter plate

	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	U51	U57
D	S4	C2	L6	L11	U18	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

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)

5.10 Benthic Macroinvertebrates

5.10.1 Introduction

Benthic invertebrates inhabit the sediment (infauna) or live on the bottom substrates or aquatic vegetation (epifauna) of lakes. The benthic macroinvertebrate assemblage in lakes is an important component of measuring the biological condition of the aquatic community and the overall ecological condition of the lake. Monitoring this assemblage is useful in assessing the status of the water body and detecting trends in ecological condition. Populations in the benthic assemblage respond to a wide array of stressors in different ways so that it is often possible to determine the type of stress or that has affected a macroinvertebrate assemblage (e.g., Klemm et al., 1990). Because many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, the condition of the structure and function of the macroinvertebrate assemblage is a response to exposure of cumulative disturbance.

For the Lakes Survey, the epibenthos will be the primary benthic indicator. Benthos are collected using a semi-quantitative sampling of multiple habitats in the littoral zone of lakes using a D-frame dip net. The lake littoral zone is made up of many microhabitat types, which have a strong influence on the macroinvertebrate assemblage. Therefore, sample collection is stratified on the following three specific habitat types: rocky/cobble/large woody debris; macrophyte beds; and organic fine muds. Targeted components of the macroinvertebrate assemblage for these habitat types are rocky-littoral epibenthos, macrophytic epibenthos, and muddy-littoral epi- and infaunal benthos, respectively.

2/7/2002 **Microcystin Immunoassay (IMN)**

Sample Tube #	Project Code	Lab ID (MT#)	Calculated* Conc. (µg/L)	Dilution	Analysis Date	Plate Name	Remarks	Tech ID	Data Entry
1		SO	0	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
2		SO	0	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
3		S1	0.15	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
4		S1	0.15	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
5		S2	0.4	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
6		S2	0.4	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
7		S3	1	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
8		S3	1	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
9		S4	2	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
10		S4	2	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
11		S5	5	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
12		S5	5	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
13		Control	0.75	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
14		Control	0.75	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
15	RRM	43340A	8.2	1:10	1/20/2005	N20JAN05		KO	
16	RRM	43340L	8.3	1:10	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
17	RRM	43388A	2.11	1:1	1/20/2005	N20JAN05		KO	
18	RRM	43389A	2.19	1:1	1/20/2005	N20JAN05		KO	
19	RRM	43390A	1.2	1:1	1/20/2005	N20JAN05		KO	
20	RRM	43391A	0.95	1:1	1/20/2005	N20JAN05		KO	
21	RRM	43391L	<0.10	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
22	RRM	43392A	<0.10	1:1	1/20/2005	N20JAN05		KO	
23	RRM	43393A	<0.10	1:1	1/20/2005	N20JAN05		KO	
24	RRM	43394A	<0.10	1:1	1/20/2005	N20JAN05		KO	
25	RRM	43394S	0.76	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
26		CCV 0.75 ppb	0.73	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
27	RRM	43395A	<0.10	1:1	1/20/2005	N20JAN05		KO	
28	RRM	43395L	<0.10	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
29	RRM	43396A	8.2	1:10	1/20/2005	N20JAN05		KO	
30	RRM	43397A	8.2	1:10	1/20/2005	N20JAN05		KO	
31	RRM	43398A	0.75	1:1	1/20/2005	N20JAN05		KO	
32	RRM	43399A	<0.10	1:1	1/20/2005	N20JAN05		KO	
33	RRM	43399L	<0.10	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
34	RRM	43401A	<0.10	1:1	1/20/2005	N20JAN05		KO	
35	RRM	43402A	<0.10	1:1	1/20/2005	N20JAN05		KO	
36	RRM	43403A	<0.10	1:1	1/20/2005	N20JAN05		KO	
37	RRM	43404A	<0.10	1:1	1/20/2005	N20JAN05		KO	
38	RRM	43405A	0.69	1:1	1/20/2005	N20JAN05		KO	
39	RRM	43405S	1.49	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	
40		CCV 0.75 ppb	0.73	1:1	1/20/2005	N20JAN05	QC sample; DO NOT ENTER	KO	

* the calculated concentration incorporated any dilutions to give the final concentration of the original sample.
Concentration range for this analysis is 0.10 – 5.0 µg/L without dilution.

Figure 5-6. Example data entry form for microcystins.

5.10.2 Sampling Design

Benthic macroinvertebrates are collected from the dominant habitat within the littoral zone of each of the 10 P-Hab stations established along the shoreline. A composite sample of macroinvertebrates is prepared from a multi-habitat approach and consists of three specific habitat types: (1) rocky/cobble/large woody debris; (2) macrophyte beds; and (3) organic fine muds or sand. The response design for sampling locations is shown in Figure 5-1.

5.10.3 Sampling and Analytical Methods

Sample Collection: Benthic macroinvertebrates are collected from the dominant habitat type within each of 10 P-Hab stations. Samples are collected using a modified D-frame kick-net (500 µm mesh) procedure and are combined together to produce a single composite sample for the lake. Samples are field-processed to remove large detritus and preserved in 70% ethanol. Detailed sampling and processing procedures are described in section 5.4 of the Field Operations Manual. A condensed description of key elements of the field activities is provided for easy reference onsite.

Analysis: Preserved composite samples are sorted, enumerated, and invertebrates identified to the lowest possible taxonomic level (generally genus, see Laboratory Methods Manual) using specified standard keys and references. Processing and archival methods are based on standard practices. Detailed procedures are contained in the laboratory operations manual and cited references. There is no maximum holding time associated with preserved benthic invertebrate samples. Table 5-31 summarizes field and analytical methods for the benthic invertebrates indicator.

5.10.4 Quality Assurance Objectives

Measurement quality objectives (MQOs) are given in Table 5-32. General requirements for comparability and representativeness are addressed in Section 2. The MQOs given in Table 5-33 represents the maximum allowable criteria for statistical control purposes. Precision is calculated as percent efficiency, estimated from examination of randomly selected sample residuals by a second analyst and independent identifications of organisms in randomly selected samples. The MQO for picking accuracy is estimated from examinations (repicks) of randomly selected residues by experienced taxonomists.

Table 5-31. Field and laboratory methods: benthic indicator

Variable or Measurement	QA Class	Expected Range and/or Units	Summary of Method	References
Sample Collection	C	NA	One-man D-frame kick net (500µm mesh) used to collect organisms, which are composited from 10 stations	Kamman 2005 (Draft); Lakes Survey Field Operation Manual 2006

Sorting and Enumeration	C	0 to 500 organisms	Random systematic selection of grids with target of 500 organisms from sample	Lakes Survey Benthic Laboratory Methods 2006
Identification	C	genus	Specified keys and references	

C = critical, N = non-critical quality assurance classification.

Table 5-32. Measurement data quality objectives: benthic indicator

Variable or Measurement	Precision	Accuracy	Completeness
Sort and Pick	95%	90%	99%
Identification	85%	90%[a]	99%

NA = not applicable
[a] Taxonomic accuracy, as calculated using Equation 9 in Section 2.

5.10.5 Quality Control Procedures: Field Operations

Specific quality control measures are listed in Table 5-34 for field operations. Additionally, duplicate (replicate) samples will be collected at 10% of lakes sampled.

5.10.6 Quality Control Procedures: Laboratory Operations

Specific quality control measures are listed in Table 5-34 for laboratory operations.

5.10.7 Data Management, Review, and Validation

Checks made of the data in the process of review, verification, and validation are summarized in Table 5-33. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members. Once data have passed all acceptance requirements, computerized data files are prepared in a format specified for the Lakes Survey project by EMAP and copied onto a floppy diskette. The diskettes are transferred to the Lakes Survey IM Coordinator at WED-Corvallis for entry into a centralized data base. A hard copy output of all files accompanies each diskette.

A reference specimen collection is prepared as new taxa are encountered in samples. This collection consists of preserved specimens in vials and mounted on slides and is provided to the responsible EPA laboratory as part of the analytical laboratory contract requirements. The reference collection is archived at the responsible EPA laboratory or other suitable facility.

Sample residuals, vials, and slides are archived by each laboratory until the EPA Project Leader has authorized, in writing, the disposition of samples. All raw data (including field data forms and bench data recording sheets) are retained permanently in an organized fashion by the Indicator Lead in accordance with EPA records management policies.

Table 5-33. 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 ≥90%	If <90%, examine all residuals of samples by that analyst and retrain analyst
Sorted samples sent to independent 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
IDENTIFICATION			
Duplicate identification by different taxonomist within lab	10% of all samples completed per laboratory	Efficiency ≥85%	If ≤85%, re-identify all samples completed by that taxonomist
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
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

6.0 FIELD AND BIOLOGICAL LABORATORY QUALITY EVALUATION AND ASSISTANCE VISITS

No national program of accreditation for phytoplankton, zooplankton, sediment diatom, algal toxin, or benthic macroinvertebrate collection and sample processing currently exists. However, national standards of performance and audit guidance for biological laboratories are being considered by the National Environmental Laboratory Accreditation Conference (NELAC). For this reason, a rigorous program of field and laboratory evaluation and assistance visits has been developed to support the Survey of the Nation's Lakes Program.

Procedural review and assistance personnel are trained to the specific implementation and data collection methods detailed in the Lakes Survey Field Operations Manual. Plans and checklists for field evaluation and assistance visit have been developed to reinforce the specific techniques and procedures for both field and laboratory applications. The plans and checklists are included in this section and describe the specific evaluation and corrective actions procedures.

It is anticipated that evaluation and assistance visits will be conducted with each Field Team early in the sampling and data collection process, and that corrective actions will be conducted in real time. These visits provide a basis for the uniform evaluation of the data collection techniques, and an opportunity to conduct procedural reviews as required to minimize data loss due to improper technique or interpretation of program guidance. Through uniform training of field crews and review cycles conducted early in the data collection process, sampling variability associated with specific implementation or interpretation of the protocols will be significantly reduced. The field visits evaluations, while performed by a number of different supporting collaborator agencies and participants, will be based on the uniform training, plans, and checklists. This review and assistance task will be conducted for each unique crew collecting and contributing data under this program; hence no data will be recorded to the project database that were produced by an 'unaudited' process, or individual.

Similarly, laboratory evaluation and assistance visits will be conducted early in the project schedule and soon after sample processing begins at each laboratory to ensure that specific laboratory techniques are implemented consistently across the multiple laboratories generating data for the program. Laboratory evaluation and assistance visit plans and checklists have been developed to ensure uniform interpretation and guidance in the procedural reviews. These laboratory visits are designed such that full corrective action plans and remedies can be implemented in the case of unacceptable deviations from the documented procedures observed in the review process without recollection of samples.

The Field and Laboratory Evaluation and Assistance Visit Plans are as follows:

6.1 Field Quality Evaluation and Assistance Visit Plan for the Survey of the Nation's Lakes (Lakes Survey)

Evaluators: One or more designated EPA or Contractor staff members who are qualified (i.e., have completed training) in the procedures of the Lakes Survey field sampling operations.

To Evaluate: Regional Monitoring Coordinator-appointed Field Sampling Teams during sampling operations on site.

Purpose: To identify and correct deficiencies during field sampling operations.

1. Tetra Tech project staff will review the Field Evaluation and Assistance Visit Plan and Check List with each Evaluator during field operations training sessions.

2. The Tetra Tech QA Officer or authorized designee will send a copy of the final Plan and 4-part carbonless copy versions of the final Check List pages, envelopes to return the Check Lists, a clipboard, pens, and Lakes Survey QAPP and *Field Operations Manua*l to each participating Evaluator.

3. Each Evaluator is responsible for providing their own field gear sufficient to accompany the Field Sampling Teams (e.g., protective clothing, sunscreen, insect repellent, hat, water bottle, food, back pack, cell phone) during a complete sampling cycle. Schedule of the Field visits will be made by the Evaluator in consultation with the Tetra Tech QA Officer and respective Field sampling crew Leader. **Evaluators should be prepared to spend additional time in the field if needed (see below).**

4. Tetra Tech and the Regional Coordinators will arrange the schedule of visitation with each Field Team, and notify the Evaluators concerning site locations, where and when to meet the team, and how to get there. Ideally, each Field Team will be evaluated within the first two weeks of beginning sampling operations, so that procedures can be corrected or additional training provided, if needed. GLEC or EPA Evaluators will visit Tetra Tech Field Teams and Tetra Tech or EPA Evaluators will visit GLEC Field Teams. Any EPA or Contractor Evaluator may visit State/Tribal Field Teams.

5. A Field Team for the Lakes Survey consists of a two- to four-person crew where, at a minimum, the Field sampling crew Leader is fully trained.

6. If members of a Field Team changes, and a majority (i.e., two) of the members have not been evaluated previously, the Field Team must be evaluated again during sampling operations as soon as possible to ensure that all members of the Field Team understand and can perform the procedures.

7. The Evaluator will view the performance of a team through one complete set of sampling activities as detailed on the Field Evaluation and Assistance Check List.

    a. Scheduling might necessitate starting the evaluation midway on the list of tasks at a site, instead of at the beginning. In that case, the Evaluator will follow the team to the next site to complete the evaluation of the first activities on the list.

    b. If the Team misses or incorrectly performs a procedure, the Evaluator will note this on the checklist and *immediately point this out so the mistake can be corrected on the spot*.

The role of the Evaluator is to provide additional training and guidance so that the procedures are being performed consistent with the *Field Operations Manual*, all data are recorded correctly, and paperwork is properly completed at the site.

c. When the sampling operation has been completed, the Evaluator will review the results of the evaluation with the Field Team before leaving the site (if practicable), noting positive practices and problems (i.e., weaknesses [might affect data quality]; deficiencies [would adversely affect data quality]). The Evaluator will ensure that the Team understands the findings and will be able to perform the procedures properly in the future.

d. The Evaluator will record responses or concerns, if any, on the Field Evaluation and Assistance Check List. They will review this list with the field sampling crew at the site.

e. If the Evaluator's findings indicate that the Field Team is not performing the procedures correctly, safely, or thoroughly, the Evaluator must continue working with this Field Team until certain of the Team's ability to conduct the sampling properly so that data quality is not adversely affected.

f. If the Evaluator finds major deficiencies in the Field Team operations (e.g., less than three members, equipment or performance problems) the Evaluator must contact one of the following QA officials:

    i. Dr. Esther Peters, Tetra Tech QA Officer (703-385-6000)
    ii. Ms. Robin Silva-Wilkinson, GLEC QA Officer (231-941-2230)
    iii. Mr. Sarah Lehmann, EPA Lakes Survey Project QA Officer (202-566-1183)

    The QA official will contact the EPA Project Leader (Ellen Tarquinio – 202-566-2267) or Alternate EPA Project Leader (Steve Paulsen – 541-754-4428) to determine the appropriate course of action.

8. Data records from sampling sites previously visited by this Field Team will be checked to determine whether any sampling sites must be redone.

9. Complete the Field Evaluation and Assistance Check List, including a brief summary of findings, and ensure that all Team members have read this and signed off before leaving the Team.

10. Retain the back copy of each page of the Field Evaluation and Assistance Check List (color: _____). Fasten the pages of the check list for each Field Team together with a paper clip.

11. Mail the remaining pages of each completed Field Evaluation and Assistance Check List to

    Dr. Esther Peters
    Tetra Tech, Inc.

10306 Eaton Place, Suite 340
Fairfax, VA 22030

12. The Tetra Tech QA Officer or authorized designee will review the returned Field Evaluation and Assistance Check Lists, note any issues, check off the completion of the evaluation for each Field Team, and distribute the remaining pages of each check list as follows:

    Original: Tetra Tech QA Officer file, Fairfax, VA

    Color: _____ Tetra Tech Project Manager file, Owings Mills, MD

    Color: _____ Lakes Survey QA Officer file, Washington, DC

6.2 Laboratory Quality Evaluation and Assistance Visit Plan for the Survey of the Nation's Lakes (Lakes Survey)

Evaluators: One or more designated Contractor staff members who are qualified (i.e., have completed training) in the procedures of the Lakes Survey laboratory operations.

To Evaluate: Laboratories performing chemical, pathogen or algal toxin analysis or subsampling, sorting, and taxonomic procedures to analyze lake samples.

Purpose: To identify and correct deficiencies during laboratory operations and procedures.

1. Tetra Tech project staff will review the Laboratory Evaluation and Assistance Visit Plan and Check List with each Evaluator prior to conducting laboratory evaluations.

2. The Tetra Tech QA Officer or authorized designee will send a copy of the final Plan and 4-part carbonless copy versions of the final Check List pages, envelopes to return the Check Lists, a clipboard, pens, and Lakes Survey QAPP and *Laboratory Methods* manual to each participating Evaluator.

3. Schedule of lab visits will be made by the Evaluator in consultation with the Tetra Tech QA Officer and the respective Laboratory Supervisor Staff. **Evaluators should be prepared to spend additional time in the laboratory if needed (see below)**.

4. Tetra Tech will arrange the schedule of visitation with each participating Laboratory, and notify the Evaluators concerning site locations, where and when to visit the laboratory, and how to get there. Ideally, each Laboratory will be evaluated within the first two weeks following initial receipt of samples, so that procedures can be corrected or additional training provided, if needed.

5. The Evaluator will view the performance of the laboratory procedures and QC Officer through one complete set of sample processing activities as detailed on the Laboratory Evaluation and Assistance Check List.

a. Scheduling might necessitate starting the evaluation midway on the list of tasks for processing a sample, instead of at the beginning. In that case, the Evaluator will view the activities of the laboratory personnel when a new sample is started to complete the evaluation of the first activities on the list.

b. If laboratory personnel miss or incorrectly perform a procedure, the Evaluator will note this on the checklist and *immediately point this out so the mistake can be corrected on the spot*. The role of the Evaluator is to provide additional training and guidance so that the procedures are being performed consistent with the *Laboratory Methods* manual, all data are recorded correctly, and paperwork is properly completed at the site.

c. When the sample has been completely processed or analyzed, the Evaluator will review the results of the evaluation with laboratory personnel and QC Officer, noting positive practices and problems (i.e., weaknesses [might affect data quality]; deficiencies [would adversely affect data quality]). The Evaluator will ensure that the laboratory personnel and QC Officer understand the findings and will be able to perform the procedures properly in the future.

d. The Evaluator will record responses or concerns, if any, on the Laboratory Evaluation and Assistance Check List.

e. If the Evaluator's findings indicate that Laboratory staff are not performing the procedures correctly, safely, or thoroughly, the Evaluator must continue working with these staff members until certain of their ability to process the sample properly so that data quality is not adversely affected.

f. If the Evaluator finds major deficiencies in the Laboratory operations, the Evaluator must contact one of the following QA officials:

   i. Dr. Esther Peters, Tetra Tech QA Officer (703-385-6000)
   ii. Mr. Dennis McCauley, GLEC QA Officer (231-941-2230)
   iii. Ms. Sarah Lehmann, EPA Lakes Survey Project QA Officer (202-566-1379)

   The QA official will contact the EPA Project Leader (Ellen Tarquinio – 202-566-2267) or Alternate EPA Project Leader (Steve Paulsen – 541-754-4428) to determine what should be done.

6. Data records from samples previously processed by this Laboratory will be checked to determine whether any samples must be redone.

7. Complete the Laboratory Evaluation and Assistance Check List, including a brief summary of findings, and ensure that the Sorter and QC Officer have read this and signed off before leaving the Laboratory.

8. Retain the back copy of each page of the Laboratory Evaluation and Assistance Check List (color: _____). Fasten the pages of the check list for each Sorter together with a paper clip.

9. Mail the remaining pages of each completed Laboratory Evaluation and Assistance Check List to:

    Dr. Esther Peters
    Tetra Tech, Inc.
    10306 Eaton Place, Suite 340
    Fairfax, VA 22030

10. The Tetra Tech QA Officer or authorized designee will review the returned Laboratory Evaluation and Assistance Check Lists, note any issues, check off the completion of the evaluation for each participating Laboratory, and distribute the remaining pages of each check list as follows:

Original: Tetra Tech QA Officer file, Fairfax, VA

Color: _____ Tetra Tech Project Manager file, Owings Mills, MD

Color: _____ Lakes Survey QA Officer file, Washington, DC

7 .0 DATA ANALYSIS PLAN

The Data Analysis Plan describes the general process used to evaluate the data for the survey. It outlines the steps taken to assess the condition of the nation's lakes and identify the relative impact of stressors on this condition. Results from the analysis will be included in the final report and used in future analysis. This is the first analysis of lakes of this scope and scale, so the data analysis plan will likely be refined and clarified as the data are analyzed by EPA and states.

7.1 Data Interpretation Background

The basic intent of data interpretation is to evaluate the occurrence and distribution of parameters throughout the population of the in the United States within the context of regionally relevant expectations for least disturbed reference conditions. This is presented using a cumulative distribution function or similar graphic. For most indicators the analysis will also categorize the condition of water as good, fair, or poor. Because of the large-scale and multijurisdictional nature of this effort, the key issues for data interpretation are unique and include: the scale of assessment, selecting the best indicators, defining the least impacted reference conditions, and determining thresholds for judging condition.

Scale of assessment. This will be the first national report on the ecological condition of the nation's lakes using comparable methods. EPA selected the sampling locations for the

survey using a probability based design, and developed rules for selection to meet certain distribution criteria, while ensuring that the design yielded a set of lakes that would provide for statistically valid conclusions about the condition of the population of lakes across the nation. A challenge that this mosaic of waterbodies poses is developing a data analysis plan that allows EPA and other partners to interpret data and present results at a large, aggregate scale.

Selecting the best indicators. Indicators should be applicable across all reporting units, and must be able to differentiate a range of conditions. As part of the indicator selection process, input from state experts at a conference co-sponsored by the Agency and the National Association of Lakes Managers, the National Conference Planning a Survey of the Nation's Lakes held April of 2005. The Agency also formed a steering committee with state and regional representatives to develop and refine indicators and sampling methodologies.

EPA developed screening and evaluation criteria which included indicator applicability on a national scale, the ability of an indicator to reflect various aspects of ecological condition, and cost-effectiveness.

Defining least impacted reference condition. Reference condition data are necessary to describe expectations for biological conditions under least disturbed setting. EPA has identified and will sample 132 reference lakes stratified by 9 ecoregions (based on Omernik Level III ecoregion) representing both natural lakes and reservoirs. EPA followed a three-step a priori screening approach proposed by Alan Herlihy under EPA cooperative agreement for identifying candidate reference lakes in four of the aggregate ecoregions - Northern Appalachians, Upper Midwest, Western Mountains, and Xeric. The approach involves screening for chemical constituents, screening with GIS coverage for landuse and road density, and screening for evidence of human disturbance based on evaluation of air photos.

For the remaining five ecoregions, EPA:

(1) Compiled lists of candidate reference lakes from regions 3-9 based on best professional judgment from the states and/or regions. In EPA Regions 1, 2 and 10, EPA had existing candidate lists. Allocation of candidate lakes to be sampled was based on natural vs. reservoir class, EPA Region, and national Ecoregion according to the below table;

(2) Examined candidate reference lakes for disturbances using aerial photographs in a 100 m buffer around the lake shoreline. Disturbances were scored from 0-3 in seven categories (residential, agricultural, recreational, industrial, forestry, water development, roads). Disturbance scores for each category were summed into one "total photo" score for use as an overall disturbance index (0 = no noted disturbances);

(3) Provided lakes with a low "total photo" scores higher preference for inclusion. EPA stratified lakes by lake surface area, Omernik level III ecoregions and then lat/long were used to spread out the sample spatially. In cases of "ties" (similar total photo scores), EPA dropped lakes with agricultural and industrial disturbances first (as opposed to road/recreation type disturbances). After that, "tie" lakes were picked randomly to fill out cells in the table. In addition to the selection of primary reference lakes, EPA listed alternates in case of

(4) limited access issues with the primary lakes. When replacing a primary lake with an alternate, EPA selected those with a similar ecoregion/lake size; and Determined the number and types of reference lakes appropriate and feasible for each region and selected reference lakes for inclusion in the 2007 sampling effort (See table below).

Allocation of Reference Lakes by EPA Region and Ecoregion

L=Natural Lake
R=Reservoir

117 Total Reference Lakes*

EPA Region	NAP	SAP	CPL	UMW	TPL	NPL	SPL	XER	WMT	TOTAL
1	20L	--	--	--	--	--	--	--	--	20
2	2L	--	--	--	--	--	--	--	--	2
3	--	10R	5R	--	--	--	--	--	--	15
4	--	--	5L	--	--	--	--	--	--	5
5	--	--	--	15L	10L	--	--	--	--	25
6	--	--	3R	--	--	--	6R	1R	--	10
7	--	5R	--	--	10R	--	10R	--	--	25
8	--	--	--	--	--	--	--	--	--	0
9	--	--	--	--	--	--	--	2L/2R	--	4
10	--	--	--	--	--	--	--	1L	10L	11
Total Lake	22	0	5	15	10	0	0	3	10	65
Total Res.	0	15	8	0	10	0	16	3	0	52

*EPA Region 1 agreed to sample 15 additional lakes bringing the total to 132 reference lakes.

Determining thresholds for judging condition. This reference site approach is then used to set expectations and benchmarks for interpreting the data on lake condition. The range of conditions found in the reference sites for an ecoregion describes a distribution of those biological or stressor values expected for least disturbed condition. The benchmarks used to define distinct condition classes (e.g., good, fair, poor / least disturbed, intermediate, most disturbed) are drawn from this reference distribution. EPA's approach is to examine the range of values for a biological or stressor indicator in all of the reference sites in a region, and to use the 5^{th} percentile of the reference distribution for that indicator to separate the most disturbed of all sites from moderately disturbed sites. Using the 5^{th} percentile means that lakes in the most disturbed category are worse than 95% of the best sites used to define reference condition. Similarly, the 25th percentile of the reference distribution can be used to distinguish between moderately disturbed sites and those in least disturbed condition. This means that lakes reported as least disturbed are as good as 75% of the sites used to define reference condition.

7.2 Datasets to be Utilized for the Report

The datasets available for use in the report were developed base on analytical methods selected during the NLA data analysis workshop. Many of the analytical methods used in the

survey stem from discussions, input, and feedback provided by the Survey of the Nation's Lakes Steering Committee. Many of the methods are an outgrowth of the testing and refinement of the existing and developed methods and the logistical foundation constructed during the implementation of the Environmental Monitoring and Assessment Program (EMAP) studies from 1991 through 1994, from a New England pilot study conducted in 2005, from focused pilot studies for methods development, and from various State water quality agency methods currently in use.

The survey will use indicators to assess trophic status, ecological integrity, and the recreational value of lakes:

Trophic status. Lakes are typically classified according to their trophic state. Three variables, chlorophyll, Secchi disk depth, and total phosphorus, are most often used to estimate biomass and define trophic state of a particular lake. Other variables will be measured in conjunction with the trophic state variables to supplement and enhance understanding of lake processes that affect primary productivity.

Ecological integrity. Ecological integrity describes the ecological condition of a lake based on different assemblages of the aquatic community and their physical habitat. The indicators include plankton (phytoplankton and zooplankton), benthic macroinvertebrates, diatoms, and the physical habitat of the shoreline and littoral zone.

Recreational value. Recreational indicators address the ability of the population to support recreational uses such as swimming, fishing and boating. The protection of these uses is one of the requirements in the Clean Water Act under 305(b). Both the extent of a fecal indicator (*Enterococci*), algal toxins (microcystin), and mercury will serve as the primary indicators of recreational value.

7.3 Trophic Status

Trophic state of lakes will be analyzed using chlorophyll-*a* concentrations, which is considered the most accurate estimator of tropic state. Trophic state will be assessed using chlorophyll-*a* concentration thresholds, as follows: oligotrophic, <2 µg/L; mesotrophic, 2 to 7 µg/L; eutrophic, 7 to <30 µg/L; and hypereutrophic, ≥30 µg/L. These categories will be used to rank the condition of lakes relative to there trophic state.

7.4 Benthic Macroinvertebrate and Zooplankton Assemblages

Benthic macroinvertebrate and zooplankton assemblage will be analyzed using both multimetric indices (MMI) and observed/expected indices (O/E) models. The MMI approach summarizes various assemblage attributes, such as composition, tolerance to disturbance, trophic and habitat preferences, as individual metrics or measures of the biological community. Candidate metrics are evaluated for aspects of performance and a subset of the best performing metrics are combined into an index known as a Macroinvertebrate Index of Biotic Condition. This index is then used to rank the condition of the resource.

The predictive model or O/E approach estimates the expected taxonomic composition of an assemblage in the absence of human stressors, using a set of least-disturbed sites and other variables related to natural gradients, such as elevation, lake size, latitude and longitude. The resulting models are then used to estimate the expected taxa composition (taxa richness) at each site sampled. The number of expected taxa actually observed at a site is compared to the number of expected taxa as an Observed Expected ratio or index. Departures from a ratio of one indicate that the taxonomic composition in the sample differs from that expected under least disturbed conditions. The greater the departure from one, the greater the sample differs from the least disturbed condition

7.5 Phytoplankton Assemblages

Phytoplankton will be collected as an integrated sample in open water. Both abundance and biovolumn on a species-specific basis will be determined. The raw data will be used in multiple data analysis techniques, metrics and indices, such as Centrales/ Pennales ratios, Palmer's WQ Index and other diversity indices.

7.6 Diatom Data Analysis

Sediment diatoms will be sampled in the deepest part of the lake (up to 50 meters), or the midpoint of a reservoir, using a sediment core sampling device. Diatoms will be analyzed/ identified in the sediment surface fraction and in a deep fraction, i.e., 35 to 45cm. Comparison of these fractions provide an indication of both current and historical lake condition with respect to stressors such as nutrients (phosphorus) and sediment loadings. Comparison of the diatoms found in deep and surface fractions can also provide insight to the structure and composition of algal communites under pristine conditions as well as inform on the temporal and spatial trends of eutrophication.

7.7 Mercury Data Analysis

Mercury levels (total and methyl) will be determined from the sediment core samples to compare to existing national mercury distribution databases. These samples will be collected from the sediment diatom core samples, from the upper portion of the core. Comparisons will be made among lakes, relative to there mercury concentrations.

7.8 *Enterococci* Data Analysis

The presence of certain levels of *enterococci* is associated with pathogenic bacterial contamination of the resource. A single *enterococci* water sample will be collected at each lake, then filtered, processed, and analyzed using quantitative Polymerase Chain Reaction,(qPCR). Bacterial occurrence and distribution will be reported. Data interpretation will be enhanced by comparison to USEPA qPCR pilot studies as well as to thresholds recommended from the Great Lakes qPCR studies. In addition, some states are doing parallel studies with better known culturing techniques that have a vast historical database which to compare.

7.9 Water Chemistry, Chlorophyll-a and Secchi Depth

A wide array of water chemistry parameters will be measured, such as DO, pH, total N, total P, clarity, TOC/DOC, color, ANC and primary productivity. Values for these parameters and their distribution will be reported. Water chemistry analysis is critical for interpreting the biological indicators. Chlorophyll-a, Secchi depth and nutrient measurements will be used to determine trophic level indices, such as the Carlson Index. Temperature profiles will be used to determine degree of lake stratification.

7.10 Algal Toxin Data Analysis

Cyanobacterial (blue-green algal) blooms are common midsummer to late-fall events that occur in many lakes and reservoirs throughout the United States. Algal toxin production has been identified as a significant potential human health problem that has been associated with many of these bloom events. However, little is known about the general occurrence of algal toxins in the pelagic zones of these water bodies, where extensive blooms are less likely to occur than in near-shore areas.

The USGS Kansas Water Science Center will analyze the total (whole water) concentrations of microcystins (total) in lakes and reservoirs throughout the United States using a standardized immunoassay test. The USGS will also perform quantitative LC/tandem MS analysis of 2% of the samples for microcystin for verification of immunoassay results. This data will be used to verify the immunoassay results and support the scientific integrity of the data.

The USGS will analyze and interpret the data for microcystin occurrence and concentration and with respect to other environmental data that is collected as part of the lake assessment (e.g. nutrients, phytoplankton, chlorophyll, turbidity, specific conductance, pH). Data interpretation by the USGS will be reviewed by the EPA and accepted through a letter of concurrence.

7.11 Physical Habitat Assessment

Shoreline human disturbances

The presence or absence of 12 predefined types of human land use or disturbance was recorded for each of the 10 stations. As part of the NLA Study, additional human disturbances were separately identified outside of but adjacent to the plots. For each of the 12 disturbance categories, We will calculate the proportion of lakeshore stations where the disturbance is observed on each lake. Proportions will be weighted according to the proximity of the disturbance before computing the whole-lake metrics. Weightings will be 1.0 for disturbance observations within the riparian sample plots and 0.33 for those behind or adjacent to the plots. Two types of summary metrics will be calculated by synthesizing all the human disturbance observations. The first, a measure of the extent of shoreline disturbance, will be calculated as the proportion of stations at which one or more human disturbances were observed. The second, a measure of disturbance intensity, will be calculated as the mean number of human disturbance types observed at each of the 10 shoreline stations.

Riparian vegetation

Riparian vegetation type and areal cover will be visually estimated in three layers: the canopy (>5 m high), mid-layer (0.5–5 m high) and ground cover (<0.5 m high). Coniferous and deciduous vegetation is distinguished in the canopy and mid-layer; woody and herbaceous vegetation was distinguished in the mid-layer and ground cover. Cover will be estimated in four classes: absent (0), sparse (0-10%), moderate (10-40%) and heavy (>40%). Another cover class was added to improve precision and interpretation, redefining "heavy" as 40-75% and "very heavy" as >75%.

Simple whole-lake metrics were calculated by assigning the cover class mid-point value to each station's observations and then averaging those cover values across all 10 stations. Summary metrics were calculated for each lake by summing the areal cover or tallying the presence of defined combinations of riparian vegetation layers or vegetation types.

Aquatic macrophytes

Using the same cover classes as for riparian vegetation, areal covers of nearshore emergent, floating, and submerged aquatic macrophytes were each estimated visually. In 1993, the same cover class redefinition was applied in aquatic macrophytes as was used for riparian vegetation. Simple and summary aquatic macrophyte metrics were calculated for each lake in the same fashion as for riparian vegetation.

Fish concealment features

The presence or absence of eight specified types of fish concealment features will be recorded within each 10-m × 15-m littoral plot. The areal cover of each type will be assigned to one of three cover classes (0, 0-10%, >10%). Simple metrics for each type of fish concealment feature are calculated as the proportion of littoral stations with the particular concealment feature present. Summary metrics will be calculated as the mean number of concealment types per station. We will use the areal cover class designation to unweight very sparse cover in the calculation of both simple and summary fish cover metrics (i.e., the areal cover designations in the previous paragraph were respectively assigned values of 0, 0.2, and 1.0).

Shoreline and littoral bottom substrate

Visual estimates of areal cover of seven defined substrate types will be made separately for the 1-m shoreline band and the bottom within the 10-m × 15-m littoral plot. Cover classes are the same as for riparian vegetation, with the same modification to include an additional higher cover class. In cases where the bottom substrate can not be observed directly, observers will use a clear plastic viewing bucket, a 3-m plastic (PVC) sounding tube, or an anchor to examine or obtain samples of bottom sediments.

Simple metrics describing the lakewide mean cover of littoral and shoreline substrate in each size category will be obtained by averaging the cover estimates at each station, using the cover class midpoint approach described for riparian vegetation. Three substrate summary metrics will be calculated for both shoreline and littoral bottom substrates. First was the mean cover of the dominant substrate type. Second and third were measures of the central tendency and variety of substrate size. Because the size categories are approximately logrithmic, we will calculate a cover-weighted mean substrate size class and its standard deviation; we will rank

the substrate classes by size from 1 to 6, weighting them by their lakewide mean cover, and then averaging weighted cover or computing its variance across size classes.

Littoral depth, bank characteristics and other observations

Lake depth 10 m offshore will be measured using SONAR, sounding line, or sounding tube. Field crews will estimate the bank angle based on high and low water marks, the vertical and lateral range in lake water level fluctuation. They will also note the presence of water surface scums, algal mats, oil slicks, and sediment color and odor. Whole-lake metrics for littoral depth and water level fluctuations are calculated as arithmetic averages and standard deviations. For bank angle classes and qualitative observations of water surface condition, sediment color, and odor, we will calculate the proportion of stations with presence of the described features.

7.11.1 Human Disturbances in Riparian/Littoral

- *12 Simple metrics* describe presence (proportion of shore) with: buildings, commercial land use, lawns, developed parkland, roads/railroads, docks/boats, trash/landfill, seawalls/revetments, rowcrop agriculture, pasture, orchards, other human activities.
- *2 Summary metrics* describe mean number of disturbance types observed per station and proportion of shoreline with human disturbance of any type.

7.11.2 Riparian Vegetation Structure

- *10 Simple metrics* describe areal cover of trees >0.3 m DBH and <0.3 m DBH in canopy layer; woody and herbaceous vegetation in mid-layer; barren ground and woody, herbaceous, and inundated vegetation in ground cover layer.
- *6 Summary metrics* describe aggregate covers in canopy + mid-layer, woody vegetation in canopy + mid-layer, and canopy + mid-layer + ground cover layers; presence of vegetation in canopy layer; presence in both canopy and mid-layer.

7.11.3 Littoral Aquatic Macrophytes

- *Simple metrics* describe cover of emergent, floating, and submergent macrophytes; and presence of macrophytes lakeward from the shoreline observation plot.
- *2 Summary metrics* describe mean combined cover and proportion of shoreline with macrophytes present.

7.11.4 Shoreline and Littoral Substrate Type and Size

- *14 Simple metrics* separately describing shoreline and littoral substrate: areal cover estimates of bedrock (>4000 mm), boulder (250–4000 mm), cobble (64–250 mm), gravel (2–64 mm), sand (0.06–2.0 mm), soil or silt/clay/muck (<0.06 mm), and vegetation or woody debris (if concealing substrate).
- *6 Summary metrics* (3 for shore and 3 for littoral bottom) estimating cover-weighted mean size class, size class variance, and the areal cover of the dominant substrate type.

7.11.5 Littoral Fish Cover

- *8 Simple metrics* estimating proportion of shore zone with various fish cover types: boulder, rock ledge, brush, inundated live trees, overhanging vegetation, snags >0.3 m diameter, aquatic macrophytes, and human structures (e.g., docks, enhancement structures).
- *Summary metrics* describing the mean number of all vegetation-related, rock-related, non-anthropogenic, and all types of fish cover types.

7.11.6 Littoral Depth, Banks, and Level Fluctuations

- *7 Simple metrics* describing mean depth and depth variation among sampling station, bank angle, and apparent height and extent of vertical and horizontal lake water level fluctuations.
- *1 Summary metric* describing spatial variation of station depths on lake.

7.11.7 Miscellaneous Habitat Variables

- *7 Simple metrics* describing proportion of sampling sites with sediment odor (petrol, H2S,) sediment colors (black, brown, other), and water surface films (oil, algal mat, other).
- *1 Summary metric* describing proportion of sampling sites with surface film of any type.

8.0 REFERENCES

American Public Health Association. 2006. *Standard Methods for the Examination of Water and Wastewater.* 21st Edition. American Public Health Association, Washington, D.C.

Bain, M.B., J.T. Finn, and H.E. Booke. 1985. Quantifying stream substrate for habitat analysis studies. North American Journal of Fisheries Management 5:499-500.

Baker, J.R. and G.D. Merritt, 1990. Environmental Monitoring and Assessment Program: Guidelines for Preparing Logistics Plans. EPA 600/4-91-001. U.S. Environmental Protection Agency. Las Vegas, Nevada.

Carlson, R.E. 1977. A trophic state index for lakes. *Limnology and Oceanography* 22(2):361-369.

Chaloud, D.C., J.M. Nicholson, B.P. Baldigo, C.A. Hagley, and D.W. Sutton. 1989. *Handbook of Methods for Acid Deposition Studies: Field Methods for Surface Water Chemistry.* EPA 600/4-89/020. U.S. Environmental Protection Agency, Washington, D.C.

Charles, D. F., C. Knowles, and R.S. Davis. 2003. Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment program. Patrick Center For Environmental Research, The Academy Of Natural Sciences. Report No. 02-06

Frissell, C.A., W.J. Liss, C.E. Warren, and M.D. Hurley. 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environ. Mgmt.* 10(2): 199-214.

Garner, F.C., M.A. Stapanian, and K.E. Fitzgerald. 1991. Finding causes of outliers in multivariate environmental data. Journal of Chemometrics. 5: 241-248.

Glew, J.R., Smol, J.P. and Last, W.M. 2001. Sediment core collection and extrusion. pp. 73-105. In: Last, W.M. and Smol, J.P. [Editors]. Tracking Environmental Change Using Lake Sediments. Vol 1: Basin Analysis, Coring, and Chronological Techniques. Kluwer Academic Publishers, Dordrecht.

Hawkins, C. P., R. H. Norris, J. N. Hogue, and J. W. Feminella. 2000. Development and evaluation of predictive models for measuring the biological integrity of streams. *Ecological Applications* 10:1456-1477.

Heinz Center. 2002. *The State of the Nation's Ecosystems.* The Cambridge University Press.

Hillman, D.C., S.H. Pia, and S.J. Simon. 1987. *National Surface Water Survey: Stream Survey (Pilot, Middle Atlantic Phase I, Southeast Screening, and Episode Pilot) Analytical Methods Manual.* EPA 600/8-87-005. U.S. Environmental Protection Agency, Las Vegas, Nevada.

Hunt, D.T.E and A.L. Wilson. 1986. *The chemical analysis of water: general principles and techniques.* 2nd edition. Royal Society of Chemistry, London, England.

Kaufmann, P.R. (ed.). 1993. Physical Habitat. IN: R.M. Hughes (ed.) *Stream Indicator and Design Workshop*. EPA600/R-93/138. U.S. Environmental Protection Agency, Corvallis, Oregon.

Kaufmann, P.R., A. T. Herlihy, J.W. Elwood, M.E. Mitch, W.S. Overton, M.J. Sale, J.J.. Messer, K.A. Cougan, D.V. Peck, K.H. Reckhow, A,J, Kinney, S.J. Christie, D.D. Brown, C.A. Hagley, and H.I. Jager. 1988. *Chemical Characteristics of Streams in the Mid-Atlantic and Southeastern United States. Volume I: Population Descriptions and Physico-Chemical Relationships.* EPA 600/3-88/021a. U.S. Environmental Protection Agency, Washington, D.C.

Kirchmer, C.J. 1983. Quality control in water analysis. Environmental Science & Technology. 17: 174A-181A.

Klemm, D.J., P.A. Lewis, F. Fulk, and J.M. Lazorchak. 1990. *Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters*. EPA 600/4-90/030. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Lemmon, P.E. 1957. A new instrument for measuring forest overstory density. *J. For.* 55(9): 667-669.

Meglen, R.R. 1985. A quality control protocol for the analytical laboratory. Pp. 250-270 IN: J.J. Breen and P.E. Robinson (eds). Environmental Applications of Cehmometrics. ACS Symposium Series 292. American Chemical Society, Washington, D.C.

Peck, D. V., and R. C. Metcalf. 1991. Dilute, neutral pH standard of known conductivity and acid neutralizing capacity. Analyst 116:221-231.

Metcalf, R. C., and D. V. Peck. 1993. A dilute standard for pH, conductivity, and acid neutralizing capacity measurement. Journal of Freshwater Ecology 8:67-72.

Mulvey, M., L. Cato, and R. Hafele. 1992. *Oregon Nonpoint Source Monitoring Protocols Stream Bioassessment Field Manual: For Macroinvertebrates and Habitat Assessment*. Oregon Department of Environmental Quality Laboratory Biomonitoring Section. Portland, Oregon. 40pp.

NAPA. 2002. *Environment.gov.* National Academy of Public Administration. ISBN: 1-57744-083-8. 219 pages.

NRC. 2000. *Ecological Indicators for the Nation.* National Research Council.

Oblinger Childress, C.J., Foreman, W.T., Connor, B.F. and T.J. Maloney. 1999. New reporting procedures based on long-term method detection levels and some considerations for interpretations of water-quality data provided by the U.S. Geological Survey National Water Quality Laboratory. U.S.G.S Open-File Report 99–193, Reston, Virginia.

Overton, W.S., White, D., and Stevens, D.L. Jr. 1991. Design report for EMAP, the Environmental Monitoring and Assessment Program. EPA/600/3-91/053, U.S. Environmental Protection Agency, Washington, D.C.

Paulsen, S.G., D.P. Larsen, P.R. Kaufmann, T.R. Whittier, J.R. Baker, D. Peck, J. McGue, R.M. Hughes, D. McMullen, D. Stevens, J.L. Stoddard, J. Lazorchak, W. Kinney, A.R. Selle, and R. Hjort. 1991. EMAP - surface waters monitoring and research strategy, fiscal year 1991. EPA-600-3-91-002. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C. and Environmental Research Laboratory, Corvallis, Oregon.

Peck, D.V., J.M. Lazorchak, and D.J. Klemm (editors). 2003. Unpublished draft. Environmental Monitoring and Assessment Program – Surface Waters: Western Pilot Study Field Operations Manual for Wadeable Streams. EPA/xxx/x-xx/xxxx. U.S. Environmental Protection Agency, Washington, D.C.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish.* EPA 440/4-89/001. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Platts, W.S., W.F. Megahan, and G.W. Minshall. 1983. *Methods for Evaluating Stream, Riparian, and Biotic Conditions*. USDA Forest Service, Gen. Tech. Rep. INT-183. 71pp.

Robison, E.G. and R.L. Beschta. 1990. Characteristics of coarse woody debris for several coastal streams of southeast Alaska, USA. Canadian Journal of Fisheries and Aquatic Sciences 47(9):1684-1693.

Robison, E.G., and P.R. Kaufmann. (In preparation). Evaluating and improving and objective rapid technique for defining pools in small wadeable streams.

Selle, A.R., D.P. Larsen, S.G. Paulsen. 1991. GIS procedure to create a national lakes frame for environmental monitoring. In: Proceedings of the 11th Annual Environmental Systems Research Institute User Conference; 1991 May 20-24; Palm Springs, CA. Corvallis, OR: U.S. Environmental Protection Agency, Environmental Research Laboratory.

Skougstad, M.W., M.J. Fishman, L.C. Friedman, D.E. Erdman, and S.S. Duncan (eds.). 1979. Method I-4600-78, Automated Phosphomolybdate Colorimetric Method for Total Phosphorus. *Methods for Determination of Inorganic Substances in Water and Fluvial Sediments: Techniques of Water-Resources Investigations of the United States Geological Survey*. Book 5, Chapter A1. U.S. Government Printing Office, Washington, D.C.

Smith, F., S. Kulkarni, L. E. Myers, and M. J. Messner. 1988. Evaluating and presenting quality assurance data. Pages 157-68 in L.H. Keith, ed. ACS Professional Reference Book. Principles of Environmental Sampling. American Chemical Society, Washington, D.C.

Stanley, T.W., and S.S. Verner. 1986. The U.S. Environmental Protections Agency's quality assurance program. pp. 12-19 IN: J.K. Taylor and T.W. Stanley (eds.). Quality Assurance for Environmental Measurements. ASTM STP 867, American Society for Testing and Materials, Philadelphia, Pennsylvania.

Stapanian, M.A., F.C. Garner, K.E. Fitzgerald, G.T. Flatman, and J.M. Nocerino. 1993. Finding suspected causes of measurement error in multivariate environmental data. Journal of Chemometrics. 7: 165-176.

Stevens, D. L., Jr., 1994. Implementation of a National Monitoring Program. Journal Environ. Management 42:1-29.

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.

U.S. EPA 2002 Guidance for Quality Assurance Plans EPA240/R-02/009 U.S. Environmental Protection Agency, Office of Environmental Information, Washington, D.C.

U.S. EPA. 2003. *Draft Report on the Environment*. ORD and OEI. EPA-260-R-02-006.

U.S. EPA. 2004. *Revised Assessment of Detection and Quantitation Approaches*. EPA-821-B-04-005. U.S. Environmental Protection Agency, Office of Science and Technology, Washington, D.C.

U.S. EPA. 2006. Guidance on Systematic Planning Using the Data Quality Objectives Process. EPA/240/B-06/001. U.S. Environmental Protection Agency, Office of Environmental Information, Washington, D.C.

USEPA. 2007. Survey of the Nation's Lakes. Field Operations Manual. EPA 841-B-07-004. U.S. Environmental Protection Agency, Washington, DC.

U.S.GAO. 2000. *Water Quality*. GAO/RCED-00-54.

Washington, H.G. 1984. Diversity, biotic, and similarity indices. *Water Research* 18(6): 653-694.

APPENDIX A

NATIONAL LAKES SURVEY FIELD EVALUATION AND ASSISTANCE SITE VISIT CHECKLIST

NATIONAL LAKES SURVEY FIELD EVALUATION AND ASSISTANCE SITE VISIT CHECKLIST

Evaluation Date(s):		
Evaluation Team Member(s):		
Name	Organization	Phone
Lake ID:	Lake Name:	
Location:		
Field Team ID:		
Field Team Members:		
Name	Organization	Phone
Other Observers Present During Evaluation:		
Name	Organization	Phone

BASE SITE ACTIVITIES			
Global Positioning System Receiver			
Were the batteries checked?	**Y**	**N**	**N/A**
Was a re-initialization check required?	**Y**	**N**	**N/A**
Were other tests or checks required as recommended in operating manual?	**Y**	**N**	**N/A**
Multi-Probe			
Was the electrode stored properly?	**Y**	**N**	**N/A**
Were the meter red lines, zeroes, readings steady?	**Y**	**N**	**N/A**
Membrane inspection: temperature, DO and pH checks conducted correctly before using?	**Y**	**N**	**N/A**
Was the DO calibration done at the lake (in accordance with 3.1.2)?	**Y**	**N**	**N/A**
Was the multi-probe calibrated for pH and conductivity at the base location or before traveling to the site (whichever is appropriate for the unit)?	**Y**	**N**	**N/A**
Was pH and conductivity (if measured) checked for performance against a QCCS solution (at the beginning of the week whenever sampling is occurring, as described in the field manual, minimum of 2x, before first and after last lake sampled)?	**Y**	**N**	**N/A**
Containers/Labels			
Were labels affixed to containers when required?	**Y**	**N**	**N/A**
Were labels completed where feasible and appropriate (before or after collection) using a permanent marker (pencil for benthos inside jar label) and covered with clear tape?	**Y**	**N**	**N/A**
Preservatives and Other Solutions			
Were stock preservatives prepared if required (recipes available)?	**Y**	**N**	**N/A**

Were the benthic invertebrate and zooplankton preservatives ready for transport?	**Y**	**N**	**N/A**
Was the preservative for phytoplankton ready for transport?	**Y**	**N**	**N/A**
Was dry ice present?	**Y**	**N**	**N/A**
Was the pH/conductivity quality control check sample solution ready for transport?	**Y**	**N**	**N/A**
Other Equipment and Supplies			
Was the current version of the Lake Visit Checklist used at the base location?	**Y**	**N**	**N/A**
Was the Supply Needs List sent or phoned in to "home base" or directly to the Field Logistics Coordinator?	**Y**	**N**	**N/A**
Were additional "custom" items added to the checklist?	**Y**	**N**	**N/A**
Were equipment and supplies clean, in verified working order, and organized for transport?	**Y**	**N**	**N/A**
Site Information and Access			
Were individual site packets, including directions to the site and topographic maps, available and organized?	**Y**	**N**	**N/A**
Was the site access information/permission letter available?	**Y**	**N**	**N/A**
Was the landowner is contacted prior to site visit?	**Y**	**N**	**N/A**
Were other key contact persons notified (e.g., Regional Coordinator, State or Tribal contacts)?	**Y**	**N**	**N/A**
Vehicle			
Was the tire pressure checked and OK?	**Y**	**N**	**N/A**
Was the fuel level checked and OK?	**Y**	**N**	**N/A**
Were the vehicle lights, turn signals, and brake lights checked?	**Y**	**N**	**N/A**
Were there any operational problems?	**Y**	**N**	**N/A**
Were emergency kit-jumper cables, first aid kit, etc. available?	**Y**	**N**	**N/A**
Was there an extra set of keys for the vehicle available and with a different person?	**Y**	**N**	**N/A**
Boat			
Was the trailer and hitch inspected prior to departing to the site to ensure that the trailer was securely fastened?	**Y**	**N**	**N/A**
Were the electronic connection and brake lights for the trailer checked?	**Y**	**N**	**N/A**
Was the boat(s) in good working order and inspected before departure?	**Y**	**N**	**N/A**
Was there any additional emergency equipment (e.g., shovel, fire extinguisher, etc.)?	**Y**	**N**	**N/A**
Were PFDs available for all passengers?	**Y**	**N**	**N/A**

Conductivity (OPTIONAL)			
Was the QC check conducted correctly before field measurement, using a DI water rinse, rinse bottle, and test bottle of QC solution?	**Y**	**N**	**N/A**
Was the measured conductivity of QCC solution recorded?	**Y**	**N**	**N/A**

Does the crew understand what to do in case of an unacceptable QC check?	**Y**	**N**	**N/A**
Was the temperature of the solution recorded (if meter does not provide temperature-corrected values)?	**Y**	**N**	**N/A**
Was the QC solution recently replaced? (2 - 3 weeks)?			
Was the conductivity measurement made at a representative location within the stream (near X-site, flowing water, mid-depth, etc.)?			
Was a measured conductivity value recorded correctly on the field form?			
Were the meter and probe stored correctly after use?			

NOTES

LAKE VERIFICATION			
Lake Verification at the Launch Site			
Was the site information sheet available for the lake?	**Y**	**N**	**N/A**
Were the lake coordinates recorded on the verification form?	**Y**	**N**	**N/A**
Was a detailed description of the final part of the route to the lake recorded?	**Y**	**N**	**N/A**
Was the lake classified correctly (e.g., target vs. nontarget vs. inaccessible)?	**Y**	**N**	**N/A**
Was the Verification Form completed for sites not visited and for sites visited but not sampled?	**Y**	**N**	**N/A**
Was a rough sketch of lake outline available for Side 2 of verification form?	**Y**	**N**	**N/A**
Does the map sketch of the lake outline include shoreline station locations and launch site location?	**Y**	**N**	**N/A**
Lake Verification at the Index Site Location			
Was the lake verified via GPS coordinates or map information and recorded on the verification form?	**Y**	**N**	**N/A**
Was the lake evaluated to see if it meets study requirements (e.g., > 1m deep)	**Y**	**N**	**N/A**
Was the deepest point, or index location, (< 50 m) determined using sonar or bathymetric map?	**Y**	**N**	**N/A**
Were the GPS coordinates of index location recorded on the form?	**Y**	**N**	**N/A**
Were photographs of the site taken (if appropriate)?	**Y**	**N**	**N/A**
Was the index site location marked on the lake outline map?	**Y**	**N**	**N/A**

NOTES

INDEX SITE SAMPLING			
Temperature, Dissolved Oxygen, and pH			
Was the depth measured at the index location, and the intervals calculated before probe was placed in the water?	**Y**	**N**	**N/A**
Were the site conditions properly recorded?	**Y**	**N**	**N/A**
Was the probe calibrated during the initial site activities?	**Y**	**N**	**N/A**
Was an operation manual available for the meter?	**Y**	**N**	**N/A**
Were the measurements at each depth interval conducted and recorded according to the protocol on the Lake Profile Form?	**Y**	**N**	**N/A**
Did the probe touch the bottom of the lake?	**Y**	**N**	**N/A**
Was a duplicate reading taken at the surface after the profile was completed?	**Y**	**N**	**N/A**
Was the probe stored correctly after the measurement?	**Y**	**N**	**N/A**
Was the top and bottom of the metalimnion marked on the form where the water temperature changes 1 degree per meter?	**Y**	**N**	**N/A**

Secchi Disk Transparency			
Was the Secchi disk being used the black and white patterned disk?	**Y**	**N**	**N/A**
Was the calibrated sounding line visibly marked in half meter intervals?	**Y**	**N**	**N/A**
Was the measurement taken from the shady side of the boat?	**Y**	**N**	**N/A**
Was the recorder wearing sunglasses or a hat?	**Y**	**N**	**N/A**
Was a viewscope used?	**Y**	**N**	**N/A**

Water Sample Collection and Preservation			
Were gloves worn?	**Y**	**N**	**N/A**
Was the integrated sampler rinsed three times at the index point?	**Y**	**N**	**N/A**
Was the euphotic zone correctly defined by the team based on Secchi depth measurements?	**Y**	**N**	**N/A**
Was the euphotic zone calculated on lake index site sample collection form?	**Y**	**N**	**N/A**
If the euphotic zone < 2m, was the sample collected from within the euphotic zone only?	**Y**	**N**	**N/A**
Were labels for all containers securely attached and covered with clear tape?	**Y**	**N**	**N/A**
Was the Lake ID correctly labeled on each container?	**Y**	**N**	**N/A**
Was the cubitainer expanded by water pressure, not by inflating or pulling apart sides?	**Y**	**N**	**N/A**
Were fingers kept away from the inner surface of the cap and container opening during sample collection?	**Y**	**N**	**N/A**
Was the first cubitainer mixed thoroughly before pouring off into the 2L bottle for chlorophyll a filtration, 1L bottle for phytoplankton, and 500 ml for the microcystin sample?	**Y**	**N**	**N/A**

Water Sample Collection and Preservation			
Are the sample jars clearly labeled for each indicator?	**Y**	**N**	**N/A**
Were approximately 10ml of Lugol's added to the 1L bottle for phytoplankton preservation?	**Y**	**N**	**N/A**
Was the sample a "weak-tea" color?	**Y**	**N**	**N/A**
For the microcystin sample, was the 500 ml bottle filled with water from the 4 L cubitainer?	**Y**	**N**	**N/A**
For microcystin sample, was the bottle placed in the cooler with wet ice?	**Y**	**N**	**N/A**
Was the cubitainer placed in dark plastic bag?	**Y**	**N**	**N/A**
Was the cubitainer placed in a cooler or in a black gallon bag on ice until the site work was complete?	**Y**	**N**	**N/A**

Zooplankton Sample Collection			
Were the mesh sizes clearly marked on the two Wisconsin nets and buckets (80μm and 243 μm)?	**Y**	**N**	**N/A**
Were the nets inspected before use for holes or tears?	**Y**	**N**	**N/A**
Were the nets each attached to a line visibly marked every 0.5m?	**Y**	**N**	**N/A**
Was the net carefully lowered through the water in an upright position?	**Y**	**N**	**N/A**
Was the net stopped 0.5 m from the bottom?	**T**	**N**	**N/A**
If the lake is < 2m deep and the Secchi disk was visible at the bottom of the lake, was a second tow conducted?	**Y**	**N**	**N/A**
Was the net pulled to the surface at a steady, constant rate (about 1 ft or 0.3 m/second)?	**Y**	**N**	**N/A**
At the surface, was the net dipped into the water to rinse organisms to the cod end?	**Y**	**N**	**N/A**
Was the outside of the net carefully rinsed at the surface with a squirt bottle or similar tool?	**Y**	**N**	**N/A**
Was the second net towed from the other side of the boat or the opposite end?	**Y**	**N**	**N/A**
Was the lake ID pre-recorded on the sample label?	**Y**	**N**	**N/A**
Was the mesh size (80μm or 243 μm) used on the jar?	**Y**	**N**	**N/A**
Were the samples collected from each net mesh size treated as two, unique samples (different sample ID numbers)?	**Y**	**N**	**N/A**
Did the 500 ml bottle contain the CO2 tablets?	**Y**	**N**	**N/A**
Was EtOH water used to rinse the zooplankton from the net into the sample bottle?	**Y**	**N**	**N/A**
If the volume of zooplankton in the bucket exceeds 125 ml, was a second jar used?	**Y**	**N**	**N/A**
If so, were the jars labeled properly? (i.e., Extra jar, and 2 of 2 added)	**Y**	**N**	**N/A**
Was approximately 80 ml of ethanol added to the jar?	**Y**	**N**	**N/A**
Was the length of the tow recorded on the label and sample collection form?	**Y**	**N**	**N/A**
Was the lid wrapped in electrical tape?	**Y**	**N**	**N/A**
Was this procedure followed separately, for each net?	**Y**	**N**	**N/A**

Zooplankton Sample Collection			
Was the Sample Collection Form completed correctly for zooplankton? Does the information on the form match the information on the label for each sample?	**Y**	**N**	**N/A**

Sediment Diatom and Mercury Sample Collection			
Were the containers properly labeled for top, bottom, and sediment cores?	**Y**	**N**	**N/A**
Was the corer cleaned from the last site visit and rinsed with tap water after arrival to this lake site?	**Y**	**N**	**N/A**
Were gloves (powderless) worn throughout this procedure?	**Y**	**N**	**N/A**
Was the core extruded from an area of undisturbed sediments?	**Y**	**N**	**N/A**
Was the core 35 cm to 45cm in length?	**Y**	**N**	**N/A**
Was the water-sediment interface maintained while placing the stopper in the bottom of the corer?	**Y**	**N**	**N/A**
Was the corer kept in a vertical position while the slices are extracted?	**Y**	**N**	**N/A**
Was the total length of the core measured to the nearest 0.1 cm?	**Y**	**N**	**N/A**
Was the water at the top of the core carefully removed with a siphoning tube, so the top sediments were not disturbed?	**Y**	**N**	**N/A**
Was the crew careful to ensure that the sampling kit did not come in contact with anything other than the sediment sample?	**Y**	**N**	**N/A**
Was the sediment from the center of the core (for mercury analysis) transferred to the vial without rinsing?	**Y**	**N**	**N/A**
Was the sediment sample placed immediately on dry ice?	**Y**	**N**	**N/A**
Was the top 1 cm of the core transferred to the sample container labeled “TOP?”	**Y**	**N**	**N/A**
Was the interval recorded on the Sample Collection Form?	**Y**	**N**	**N/A**
For natural lakes, was the sectioning apparatus rinsed before the bottom slice was extracted?	**Y**	**N**	**N/A**
For natural lakes, was the sediment extruded until the bottom of the stopper was 5 cm from the top of the coring tube? Was the tube marked at 5 cm?	**Y**	**N**	**N/A**
For natural lakes, were the next 2 cm extruded and discarded?	**Y**	**N**	**N/A**
For natural lakes, was the next 1 cm extruded and kept as the “BOTTOM” slice?	**Y**	**N**	**N/A**
Was this interval correctly recorded on the Sample collection form?	**Y**	**N**	**N/A**
Were the labels secured with clear plastic tape?	**Y**	**N**	**N/A**
Was the corer cleaned and rinsed with lake water after all samples were collected?	**Y**	**N**	**N/A**

NOTES

PHYSICAL HABITAT EVALUATION			
Site Selection and Location			
Were habitat sites selected randomly and distributed evenly around the lake perimeter?	**Y**	**N**	**N/A**
Were habitat sites located accurately (using GPS, lake outline, or topography) and the plots properly lain out?	**Y**	**N**	**N/A**
Were habitat sites adjusted reasonably and only when necessary?	**Y**	**N**	**N/A**
Was the lake outline map on the verification form marked appropriately for the adjusted stations?	**Y**	**N**	**N/A**
Was an observation vantage point established at 10 m off the shore and on centerline of the plot?	**Y**	**N**	**N/A**
Was the water depth at 10 m off shore measured with a sounding or sonar and recorded accurately (including units)?	**Y**	**N**	**N/A**
Bottom Substrates			
Were bottom substrates visually observed or probed with a sounding pole throughout littoral plot?	**Y**	**N**	**N/A**
Were the categories of bottom substrates interpreted correctly?	**Y**	**N**	**N/A**
Did the categorical levels of bottom substrates potentially add up to 100%?	**Y**	**N**	**N/A**
Aquatic Macrophytes			
Were aquatic macrophytes correctly categorized and characterized?	**Y**	**N**	**N/A**
Was the total macrophyte coverage consistent with coverage in the individual categories?	**Y**	**N**	**N/A**
Fish Cover			
Were the elements of fish cover properly identified and quantified?	**Y**	**N**	**N/A**
Riparian vegetation			
Were the canopy, understory, and ground cover correctly and completely characterized?	**Y**	**N**	**N/A**
Were the vegetative types consistent with coverage categories?	**Y**	**N**	**N/A**
Shoreline Substrate Zone			
Were the shoreline substrates in the first landward meter properly identified and quantified?	**Y**	**N**	**N/A**
Human Influence			
Were the human influences properly identified within or near the plot?	**Y**	**N**	**N/A**
Littoral Fish Macrohabitat			
Were all fields complete?	**Y**	**N**	**N/A**
Were selections consistent with information on front of form?	**Y**	**N**	**N/A**
Bank Features			
Was the bank angle correctly interpreted in the first landward meter and recorded?	**Y**	**N**	**N/A**
Was the high water mark correctly identified?	**Y**	**N**	**N/A**

Were the horizontal and vertical distances from the current waterline correctly estimated or measured and recorded (in meters)?	**Y**	**N**	**N/A**
Invasives			
Were the species correctly marked or "none observed" marked in both the littoral and riparian columns?	**Y**	**N**	**N/A**
Whole Form			
Were the site and date information complete?	**Y**	**N**	**N/A**
Was one habitat form completed per station (additional forms included for new sites, e.g., islands)?	**Y**	**N**	**N/A**
Were data flags used appropriately and explained adequately throughout the form?	**Y**	**N**	**N/A**
Was the form reviewed and initialed?	**Y**	**N**	**N/A**
Were the comments legible?	**Y**	**N**	**N/A**

Benthic Macroinvertebrate Sample Collection			
After locating the sample site, was the dominant habitat type identified within the plot?	**Y**	**N**	**N/A**
Was a D-frame dip net (equipped with 500 µm mesh) used to sweep through 1 linear meter of the dominant habitat type at a single location within the 10 m x 15 m littoral zone sampling area, making sure to disturb the substrate enough to dislodge organisms?	**Y**	**N**	**N/A**
If the dominant habitat is rocky/cobble/large woody debris, did the crew member conducting the sampling exit the boat and disturb the substrate (e.g., overturn rocks, logs) using his/her feet while sweeping the net through the disturbed area?	**Y**	**N**	**N/A**
After completing the 1-meter sweep, were organisms and debris removed from net and placed in a bucket?	**Y**	**N**	**N/A**
Were the organisms and detritus collected at each station on the lake combined in a single bucket to create a single composite sample for the lake?	**Y**	**N**	**N/A**

Fecal Indicator (Enterococci) Sample Collection			
Were gloves worn?	**Y**	**N**	**N/A**
Was the sodium thiosulfate tablet transferred from the pre-sterilized, 250 ml sample bottle to a sterile screw-cap 50-ml PP tube?	**Y**	**N**	**N/A**
Was the sampling location 1 m deep and approached slowly from downstream or downwind?	**Y**	**N**	**N/A**
Was the 250 ml sample bottle lowered un-capped and inverted to a depth of 0.3 meters below the water surface, avoiding surface scum, vegetation, and substrates?	**Y**	**N**	**N/A**
Was the mouth of the container pointed away from the body or boat?	**Y**	**N**	**N/A**
Was the bottle righted and raised through the water column, allowing the bottle to fill completely?	**Y**	**N**	**N/A**

Fecal Indicator (Enterococci) Sample Collection			
After removing the container from the water, was a small portion of the sample discarded to allow for proper mixing before analyses?	**Y**	**N**	**N/A**
Was the sodium thiosulfate tablet added along with the cap, and the bottle shaken 25 times?	**Y**	**N**	**N/A**
Was the sample stored in a cooler on ice to chill (not freeze)?	**Y**	**N**	**N/A**
Was the sample chilled for at least 15 minutes and held for less than 8 hours before filtration?	**Y**	**N**	**N/A**

NOTES

FINAL LAKE ACTIVITIES			
General Lake Assessment			
Were any of the sources of potential stressors recorded that were observed while on the lake, while driving or walking through the lake catchment, or while flying over the lake and catchment?	**Y**	**N**	**N/A**
For activities and stressors that the crew observed, was their abundance or influence as low (L), moderate (M), or heavy (H) rated on the line next to the listed disturbance?	**Y**	**N**	**N/A**
Was the box on the assessment forms checked to denote blanks as zeros?	**Y**	**N**	**N/A**
Was the section "Lake Site Activities and Disturbances Observed" completed including residential, recreational, agricultural, industrial, and lake management categories?			
Were observations regarding the general characteristics of the lake recorded?	**Y**	**N**	**N/A**
Was the hydrologic lake type recorded?	**Y**	**N**	**N/A**
Were flight hazards noted that might interfere with either low-altitude fly-overs by aircraft (for future aerial photography or videography) or landing on the lake for sampling purposes (either by float plane or helicopter)?	**Y**	**N**	**N/A**
When estimating the intensity of motor boat usage, in addition to the actual number of boats observed on the lake during the visit, were other observations such as the presence of boat houses, docks, and idle craft recorded?	**Y**	**N**	**N/A**
Were all six characteristics estimated and the section "General Lake Information" completed?	**Y**	**N**	**N/A**
When the extent of major vegetation types was estimated, was the assessment limited to the immediate lake shoreline (i.e., within 20 m of the water)?	**Y**	**N**	**N/A**
Was the percentage of the immediate shoreline that has been developed or modified by humans estimated?	**Y**	**N**	**N/A**
Were all eight shoreline categories completed and the section "Shoreline Characteristics" estimated?	**Y**	**N**	**N/A**
Was the areal percentage of macrophyte coverage for the three categories estimated and the section "Qualitative Macrophyte Survey" completed?	**Y**	**N**	**N/A**
Was the waterbody character rated?	**Y**	**N**	**N/A**
Was the water body character defined by using degree of human development and aesthetics attributes?	**Y**	**N**	**N/A**
Were the three ecological values (i.e., trophic state, ecological integrity, and recreation) assessed?	**Y**	**N**	**N/A**
For ecological values, was the overall impression of the "health" of the biota in the lake recorded and note any possible causes of impairment?	**Y**	**N**	**N/A**
For tropic status, was a visual impression of the trophic status including overall impression of algal abundance and general type provided?	**Y**	**N**	**N/A**
For tropic status, were any observed potential nutrient sources to the lake listed?	**Y**	**N**	**N/A**
For recreation, was the overall impression of the lake as a site for recreation recorded?	**Y**	**N**	**N/A**

FINAL LAKE ACTIVITIES			
General Lake Assessment			
For recreation, were possible causes of impairment, or the presence or absence of people using the lake for recreational activities recorded?	**Y**	**N**	**N/A**
Was the comments section used on the Lake Assessment Form to note any other pertinent information about the lake or its catchment?	**Y**	**N**	**N/A**

NOTES

FINAL LAKE ACTIVITIES			
Processing the Fecal Indicator			
Were non-powdered surgical gloves worn?	**Y**	**N**	**N/A**
Were the Filter Extraction tubes with beads chilled on dry ice?	**Y**	**N**	**N/A**
Were the 4 PC filters aseptically transferred from the filter box to the base of the opened Petri dish?			
Was the cellulose nitrate filter removed from funnel and discarded?	**Y**	**N**	**N/A**
Was the filtration funnel loaded with sterile PC filter on the support pad (shiny side up)?	**Y**	**N**	**N/A**
Was the sample bottle(s) shaken 25 times to mix well?	**Y**	**N**	**N/A**
Was the 25 ml of the mixed water sample measured in the sterile graduated PP tube and poured into the filter funnel?	**Y**	**N**	**N/A**
Was it pumped until all liquid was in the filtrate collection flask?	**Y**	**N**	**N/A**
If the first 25 ml volume passed readily through the filter, was another 25 ml added and the filtration continued?	**Y**	**N**	**N/A**
If the filter clogged before completely filtering the first or second 25 ml volume, was the filter discarded and the filtration repeated using a lesser volume?	**Y**	**N**	**N/A**
Was a quarter (approx. 25 ml) of the chilled Dilution Buffer poured into the graduated PP tube used for the sample?	**Y**	**N**	**N/A**
Was the tube capped and shaken 5 times?	**Y**	**N**	**N/A**
Was the cap removed and the rinsate poured into the filter funnel to rinse filter?	**Y**	**N**	**N/A**
Was the rinsate filtered and repeated with another 25 ml of Dilution Buffer?	**Y**	**N**	**N/A**
Was the filter funnel removed from the base without disturbing filter?	**Y**	**N**	**N/A**
Were sterile disposable forceps used to remove the filter (touching only the filter edges)?	**Y**	**N**	**N/A**
Was the filter folded it in half, in quarters, and then in eighths?	**Y**	**N**	**N/A**
Was the filter inserted into chilled filter extraction tube (with beads)?	**Y**	**N**	**N/A**
Was the screw cap replaced and tightened?	**Y**	**N**	**N/A**
Was the tube(s) inserted into ziplock bag on dry ice for preservation during transport and shipping?	**Y**	**N**	**N/A**
Was the volume of water sample filtered through each filter recorded?	**Y**	**N**	**N/A**
If 25 ml of dilution buffer was not used, was this flagged and noted on the collection form?	**Y**	**N**	**N/A**
Was the filtration start time and finish time recorded for each sample?	**Y**	**N**	**N/A**
Were the steps repeated for the remaining three 50 ml sub-sample volumes to be filtered?	**Y**	**N**	**N/A**

NOTES

FINAL LAKE ACTIVITIES			
Processing the Chlorophyll-*a* Sample			
Were surgical gloves worn?	**Y**	**N**	**N/A**
Was a glass fiber filter placed in the graduated filter holder apparatus?	**Y**	**N**	**N/A**
Was the filter handled with forceps?	**Y**	**N**	**N/A**
Was 250 ml of water poured into the filter holder, the cap replaced, and the sample pumped through the filter?	**Y**	**N**	**N/A**
If 250 ml of lake water did not pass through the filter, was the filter changed, the apparatus rinsed with DI water, and the procedures repeated using 100 ml of lake water?	**Y**	**N**	**N/A**
Was the upper portion of the filtration apparatus rinsed thoroughly with DI water to include any remaining cells adhering to the sides and pumped through the filter?	**Y**	**N**	**N/A**
Was the level of water monitored in the lower chamber to ensure that it did not contact the filter or flow into the pump?	**Y**	**N**	**N/A**
Was the filter observed for visible color?	**Y**	**N**	**N/A**
If there was not, did the process proceed until color was visible on the filter or until a maximum of 2,000 ml was filtered?	**Y**	**N**	**N/A**
Was the actual sample volume filtered recorded on the Sample Collection Form and on the sample label?	**Y**	**N**	**N/A**
Was the bottom portion of the apparatus removed and the water poured off from the bottom?	**Y**	**N**	**N/A**
Was the filter removed from the holder with clean forceps?	**Y**	**N**	**N/A**
Was the filter folded in half, with the colored side folded inward?	**Y**	**N**	**N/A**
Was the folded filter placed into a 50 ml steam-top centrifuge tube and caped?	**Y**	**N**	**N/A**
Was the sample volume filtered recorded on a chlorophyll label and attached to the centrifuge tube?	**Y**	**N**	**N/A**
Was all written information complete and legible?	**Y**	**N**	**N/A**
Was the label covered with a strip of clear tape?	**Y**	**N**	**N/A**
Does the “total volume of water filtered” on the Sample Collection Form match the total volume recorded on the sample label?	**Y**	**N**	**N/A**
Was the tube wrapped in aluminum foil and placed in a self-sealing plastic bag?	**Y**	**N**	**N/A**
Was this bag placed between two small bags of ice in a cooler?	**Y**	**N**	**N/A**
Were the filter chambers rinsed with DI water?	**Y**	**N**	**N/A**

NOTES

FINAL LAKE ACTIVITIES			
Data Forms and Sample Inspection			
After the Lake Assessment Form was completed, did the Field Team Leader review all of the data forms and sample labels for accuracy, completeness, and legibility?	**Y**	**N**	**N/A**
Did the other team member inspect all sample containers and packages in preparation for transport, storage, or shipment?	**Y**	**N**	**N/A**
Did the team ensure that all required data forms for the lake were completed?			
Was it confirmed that the LAKE-ID and date of visit are correct on all forms?	**Y**	**N**	**N/A**
On each form, was it verified that all information was recorded accurately, the recorded information was legible, and any flags were explained in the comments section?	**Y**	**N**	**N/A**
Was it ensured that written comments are legible, with no "shorthand" or abbreviations?	**Y**	**N**	**N/A**
After reviewing each form, was the upper right corner of each page of the form initialed?	**Y**	**N**	**N/A**
Was it ensured that all samples were labeled, all labels are completely filled in, and each label was covered with clear plastic tape?	**Y**	**N**	**N/A**
Were all sample containers checked to ensure that they were properly sealed?	**Y**	**N**	**N/A**
Will the coolers be shipped with fresh bags of ice in cooler; ice bags labeled as "ICE"?	**Y**	**N**	**N/A**
Will the coolers be shipped by overnight courier ASAP after collection (generally the next day)?	**Y**	**N**	**N/A**
If samples will be held after collection, will they kept cold and in darkness?	**Y**	**N**	**N/A**
Were the Wisconsin nets and buckets rinsed at least three times with the DI water?	**Y**	**N**	**N/A**

NOTES

FINAL LAKE ACTIVITIES			
Launch Site Cleanup			
Were the boat, motor, and trailer inspected for evidence of weeds and other macrophytes?	**Y**	**N**	**N/A**
Were the boat, motor, and trailer cleaned as completely as possible before leaving the launch site?	**Y**	**N**	**N/A**
Were all nets inspected for pieces of macrophyte or other organisms and as much as possible was removed before packing the nets for transport?			
Were all equipment and supplies packed in the vehicle and trailer for transport and kept as organized as presented in the equipment checklists?	**Y**	**N**	**N/A**
Was all waste material at the launch site cleaned up and disposed of or transported it out of the site if a trash can is not available?	**Y**	**N**	**N/A**

NOTES

Miscellaneous			
Do the team members know the Communications Center phone number by heart, is the number is saved in cell phone, or do they know the location of number in Field Ops Manual?	**Y**	**N**	**N/A**
Do the team members have suggestions/problems concerning the sampling Procedures, forms, lodging, logistics, etc.?	**Y**	**N**	**N/A**

FINAL EVALUATION ACTIVITIES
Areas of Strength

FINAL EVALUATION ACTIVITIES
Areas of Concern

FINAL LAKE ACTIVITIES			
Was the team debriefed on the results of the evaluation by the evaluator?	**Y**	**N**	**N/A**
COMMENTS OF THE TEAM BEING EVALUATED			

SIGNATURES

_____	_____	_____	_____
Evaluator	Date	Field Team Leader	Date
_____	_____	_____	_____
Field QC Officer (if assigned by site)	Date	Field Team Member	Date
_____	_____	_____	_____
Field Team Member	Date	Field Team Member	Date
_____	_____	_____	_____
Field Team Member	Date	Field Team Member	Date
_____	_____	_____	_____
Field Team Member	Date	Field Team Member	Date

APPENDIX B

LAKE SURVEY LABORATORY LIST

Appendix B
Lakes Survey Laboratory List

Support	Contact	Contractor	Contractor No. & Task No.	Project Officer
Field Sampling	Michael Barbour 410-356-8993	TetraTech, Inc. 10306 Eaton Place Fairfax, VA 22030 703-385-6000	AWPD 68-C-02-108 Task No. 167	Carol Peterson EPA/OW/OWOW (4303T) 1200 Pennsylvania Ave. NW Washington, DC 20460 202-566-1304
Laboratory Water Chemistry Analysis	Dave Peck EPA/COR 541-754-4463	Dynamac Corp. c/o U.S.EPA 200 SW 35th St. Corvallis, OR 97333 541-754-4463	EP-D-06-013 Work Assignment 1-06	Kathy Martin U.S. EPA NHEERL-WED 200 S.W. 35th St. Corvallis, OR 97333 541-754-4502
Sediment Diatom Analysis	Dennis McCauley 231-941-2230	R. Jan Stevenson, Ph.D. Co-Director, Center for Water Sciences and Professor, Department of Zoology 203 Natural Science Building Michigan State University East Lansing, MI 48824 Phone: 517-432-8083 FAX: 517-432-2789 www.msu.edu/~rjstev Kociolek, Patrick Diatom Collection California Academy of Sciences 875 Howard Street San Francisco, CA 94103	HECD 68-C-04-006 Work Assignment 3-58	Carol Peterson EPA/OW/OWOW (4303T) 1200 Pennsylvania Ave. NW Washington, DC 20460 202-566-1304

		Mark A. Schadler Phycology Project Manager Patrick Center for Environmental Research Academy of Natural Sciences 1900 Benjamin Franklin Pkwy Philadelphia, PA 19103 215-299-3792		
qPCR for *enterococci* Analysis	Jack Paar 617-918-8300	TechLaw, Inc. 14500 Avion Parkway Chantilly, VA 20151-1101 703-816-1000	ESAT Contract No. EP-W-06-17 Task Order 08 (non-superfund PCR support)	Pat Svetaka U.S.EPA Regional Lab (EQA) 11 Technology Dr. N. Chelmsford, MA 01863-2431 617-918-8396
Algal Toxin Analysis	Keith Loftin 785-832-3543	USGS Kansas Water Science Center 4821 Quail Crest Place Lawrence ,KS 66049 785-832-3511	IAG No. DW 14922508-01-0	Susan Holdsworth EPA/OW/OWOW (4303T) 1200 Pennsylvania Ave. NW Washington, DC 20460 202-566-1187
Zooplankton Analysis	Ellen Tarquinio 202-566-2267	EcoAnalysts, Inc. 105 E 2nd St. Suite 1 Moscow, ID 83843 (208) 882-2588	BPA 07-03	Ellen Tarquinio EPA/OW/OWOW (4305T) 1200 Pennsylvania Ave. NW Washington, DC 20460 (202) 566-2267
Phytoplankton Analysis	Ellen Tarquinio 202-566-2267	EcoAnalysts, Inc. 105 E 2nd St. Suite 1 Moscow, ID 83843 (208) 882-2588	BPA 07-02	Ellen Tarquinio EPA/OW/OWOW (4305T) 1200 Pennsylvania Ave. NW Washington, DC 20460 (202) 566-2267