Chapter XII

QUALITY ASSURANCE / QUALITY CONTROL

A. Components and Data Quality Objectives

Quality assurance is a continual program evaluation which functions to provide data users with a given level of data confidence. In order to assure accurate data is being collected, the quality of both equipment and data collectors must be evaluated. SEARCH assures quality data is being collected through continual training of teachers and by accompanying schools on sampling trips.

Quality controls are specific activities performed during data collection which insure that data meet known accuracy and precision criteria. Quality controls include internal checks and independent testing and must cover every aspect of the data generation process, including sample design and data collection, processing, and analysis.

Five key components of a quality assurance/quality control program are: completeness, comparability, representativeness, accuracy, and precision. A description of each component as it pertains to the SEARCH program follows.

Completeness. Completeness refers to the amount of valid data collected versus the amount expected to be collected. SEARCH schools gather data on a variety of parameters in three major areas: physical, chemical, and biological (Table 12.1). Ideally, all parameters are tested during two fall and two spring field trips. Schools which have severe time and transportation constraints are expected to address a minimum of two of the following: physical parameters, chemical parameters, macroinvertebrates, and fecal coliform. Collections must be made at least once in the fall and once in the spring. The goal of the SEARCH program is to have all schools sampling 100% of the parameters. SEARCH staff provides adequate training and any necessary assistance to make SEARCH teachers proficient at sampling.

Table 12.1. Major parameters and components of SEARCH data.

Parameters	Components
Physical	Stream flow, Stream profile, Stream corridor assessment, Drainage basin mapping, Habitat assessment, Temperature, and Specific Conductivity
Chemical	pH, Dissolved Oxygen, Biochemical Oxygen Demand (5 day), Hardness (Total, Calcium, Magnesium), Alkalinity, Ammonia-N, Nitrate-N, and Orthophosphate
Biological	Benthic macroinvertebrates and Fecal coliform

Comparability. One of the major sources of bias in water quality data is the improper selection of a study site (Thornton *et al.* 1982). In order to assure that SEARCH data is comparable to both state sites and other schools, the following guidelines must be met.

1. Minimal Requirements for a Study Site

- Lotic erosional area (riffle) of 5 meters in length.

- Bankful flow during mean low flow.

- Depth is crossable year round with hipboots.

- One hundred meters from point source inputs (storm drains, lake/pond outflows, sewage effluent *etc.*).

2. Stream Habitat Assessment

- Stream Habitat Assessments are based on *Rapid Bioassessment Protocols* (Plafkin *et al.* 1989).

- All habitat assessments are conducted during representative periods of mean low-flow (June through September).

- All study sites are assessed by a single SEARCH staff member to eliminate any subjective bias. The assessment value is used to calculate percent comparability with other streams. Two sites are considered comparable if the habitat assessment point totals are within 89% of each other (Plafkin *et al.* 1989).

Representativeness. In order to assure that data collected is representative of true conditions, SEARCH has the following guidelines.

1. All samples collected during the year are taken from a single riffle area.

2. Recent (within 3 days) weather conditions are noted. Any data potentially biased by those conditions are also noted.

3. Water samples for all chemistry tests are gathered at the same time. Sampling of macroinvertebrates, fecal coliform, and flow can be done within two weeks of the chemistry collection. If all parameters are sampled on the same trip, water samples for chemistry and fecal coliform are collected prior to invertebrate sampling. Also, invertebrates are not collected in areas that have been disturbed by other sampling activities. Physical parameters such as stream profile and stream flow are measured after all other work is complete.

4. All chemistry tests are done in replicates of three. Results of any replicate that deviate +/- 10% of the mean are noted.

Precision and Accuracy. Both precision and accuracy provide information about the quality of the data to a potential user. Accuracy refers to how close sample results are to the true value. It can be expressed as a percentage by the following equation:

$$\text{Accuracy (\%)} = 100 \times \frac{\text{(True value-Sample value)}}{\text{(True value)}}$$

Precision refers to how close each data point is to another. It is measured statistically using standard deviation.

The SEARCH program requires measurement of water temperature, specific conductivity, and ten water chemistry parameters. Data quality objectives (DQO) for accuracy and precision for each of these parameters are listed in Table 12.2. SEARCH schools use DQO's as standards of perfection to strive towards.

Table 12.2. Accuracy and precision goals for the parameters collected by SEARCH schools.

PARAMETER	METHOD	SENSITIVITY	ACCURACY	PRECISION
Temperature	hand-held mercury thermometer	+/- $.5^0$ C	+/- 1^0 C	+/- $.5^0$ C
TDS	digital meter	10 ppm	+/- 2%	+/- 10 ppm
Conductivity	digital TDS meter			+/- 15 *u*S
pH	digital meter	+/- 0.1	+/- 0.5	+/- 0.2
Dissolved Oxygen	micro-titration (Winkler)	0.2 ppm	+/- 0.5 ppm	+/- 0.2 ppm
B.O.D. (5-day)	micro-titration (Winkler)	0.2 ppm	+/- 0.2 ppm	+/- 0.2 ppm
Hardness (total)	micro-titration	+/- 4 ppm	+/- 8 ppm	+/- 4 ppm
Hardness (Ca)	micro-titration	+/- 4 ppm	+/- 8 ppm	+/- 4 ppm
Hardness (Mg)	micro-titration	NA	+/- 2 ppm	NA
Alkalinity	micro-titration	+/- 4 ppm	+/- 8 ppm	+/- 4 ppm
Nitrate-N	colorimeter	+/- 0.05 ppm	+/- 0.1 ppm	+/- 0.05 ppm
Ammonia-N	colorimeter	+/- 0.05 ppm	+/- 0.1 ppm	+/- 0.05 ppm
Orthophosphate	colorimeter	+/- 0.02 ppm	+/- 0.02 ppm	+/- 0.01 ppm

B. Methods and Techniques

1. Physical Parameters

* **Stream Flow.** All flow measurements are taken in an area of laminar flow. The width of the stream is determined with a tape measure. The time it takes an orange to float downstream 10 feet is measured with a stopwatch at three different intervals across the stream. The depth at each interval is measured with a yard stick. Calculations yield discharge in cubic feet per second.

* **Stream Profile.** All measurements are taken perpendicular to flow across the riffle. Width and depths are recorded to the nearest centimeter.

* **Habitat assessment.** Habitat assessment follows guidelines in *Rapid Bioassessment Protocols* (Plafkin *et al*. 1989).

Assuring accuracy: All habitat assessment values recorded in the database are determined by a single SEARCH staff member, eliminating any subjective bias between evaluators.

* **Stream corridor assessment.** Depth stream corridor assessments follow procedures in *Rapid Bioassessment Protocols* (Plafkin *et al*. 1989).

*** Temperature**
Equipment used: A standard glass mercury thermometer.

Procedure: The thermometer is held at mid-depth in a shaded portion of the riffle and allowed to equilibrate for a minimum of 5 minutes before reading.

Assuring accuracy: At each site the thermometer is checked for bubbles. If a bubble exists, the thermometer is discarded. The temperature is read while the tip of the thermometer is still immersed.

*** Specific Conductivity**
Equipment used: An Oakton™ Total Dissolved Solids (TDS) Testr1™ hand-held meter.

Procedure: The TDS meter is held in the riffle until the reading stabilizes. Conductivity is calculated from TDS readings using the formula: Conductivity = TDS/0.67.

Assuring Accuracy: Meters are calibrated with conductivity standard solution (718umhos/cm) at least once per sampling season.

Maintenance: Batteries are replaced as necessary or every three years, which ever comes first.

2. Chemical Parameters

*** Sample Collection.** Water collections follow protocols found in Standard Methods (Greenberg *et al.* 1992). Collected samples are field stored in a cooler on ice until analyzed or brought to a lab. Any samples that cannot be analyzed in the field or at the lab are transferred to a refrigerator and stored at 4^0C. Samples are preserved according to specific test requirements and acid-washed containers are used when appropriate (Chapter VII,
Table 1).

Field Tests: In order to assure sample representativeness, the following tests must be done in the field.

*** pH**
Equipment used: LaMotte pH tester 1 (model 5-1755) hand-held meter.

Procedure: The meter is placed in a plastic beaker containing 100 ml minimum of sample water, swirled, and allowed to equalize. The procedure is repeated twice more with a new water sample.

Assuring accuracy: New meters are first conditioned by soaking in water for a minimum of one hour. Calibration to pH 7 occurs with standard buffer solution at each new site and each new sampling date.

Maintenance: Back at the lab the electrode is rinsed with distilled water. A small piece of paper or sponge is place in the cap and kept moist to prevent the electrode from drying out. Batteries are replaced as necessary or every three years, which ever comes first.

*** Dissolved Oxygen (D.O.)**
Equipment used: LaMotte kit model AG-30 code 7414.

Procedure: Collection bottles are rinsed once with sample water before sample collection. Collections are made just below the water surface by holding the collection bottle at an angle and allowing water to slowly flow down the inside. When nearly full, the bottle is submerged, tipped vertically to remove the air pocket, and capped. Any sample with air bubbles is recollected. After an adequate sample has been collected, it is "fixed" following kit directions. If the sample is to be analyzed at the lab (within 8 hours), it is stored in a cooler with ice.

Assuring accuracy: To prevent contamination by atmospheric oxygen all air bubbles are removed underwater by tapping the side of the bottle. Once all of the air bubbles are removed, the bottle is capped underwater. If any air bubbles remain after the bottle is removed from the water, the sample is collected again. Titrations are done against a white piece of paper to observe faint color changes.

Maintenance: All sample bottles are washed with distilled water and air dried after each use. Reagents are maintained as per LaMotte shelf-life recommendations. Any reagents which deviate in color from the Materials Safety Data Sheets or have formed a precipitate are discarded and replaced.

*** Biochemical Oxygen Demand (B.O.D.)**
Equipment used: Three dissolved oxygen sample bottles (LaMotte 0688-DO) covered with an opaque material to prevent penetration by sunlight.

Procedure: Samples are collected following collection procedures for Dissolved Oxygen. B.O.D. samples are placed in a cooler with ice until returned to the lab where they are placed at room temperature in a dark closet. After 5 days, a dissolved oxygen test is performed on each sample. B.O.D. is equal to the mean 5-day value subtracted from the mean original value.

Assuring accuracy: All air bubbles must be removed following dissolved oxygen procedures. Sample bottles are completely covered with an opaque material (tin foil or electrical tape) prior to or immediately after an adequate sample is taken.

Maintenance: All bottles are washed with distilled water and air dried after each use.

Remaining Chemical Tests: The following tests may be done in either in the field or back in the lab depending upon time schedules.

*** Hardness: Total, Calcium, and Magnesium**
Equipment used: LaMotte kit model PHT-CM-DR-LT code 4824DR-LT.

Procedure: Tests follow kit directions.

Assuring accuracy: The titrating tube must be placed on a level surface in order to properly align the meniscus with the sample volume line. Titrant is added **one drop at a time** and swirled to assure complete mixing. Titrations are done against a white piece of paper.
Note: If the end-point color is not the expected blue color but a grey-purple color, the test is redone adding two drops of hardness reagent number 7 (4487 DR) prior to adding the first step in the directions. The final result includes the volume of the two drops.

Maintenance: All reagents are stored and replaced as recommended by the manufacturer. All glassware is washed with distilled water and air dried after each use.

*** Alkalinity**
Equipment used: LaMotte field kit model WAT-MP-DR code 4533-DR.

Procedure: Follow LaMotte kit directions.

Assuring accuracy: The titrating tube must be placed on a level surface in order to properly align the meniscus with the sample volume line. Titrant is added **one drop at a time** and swirled to assure complete mixing. Titrations are done against a white piece of paper.

Maintenance: All reagents are stored and replaced as recommended by the manufacturer. All glassware is washed with distilled water and air dried after each use.

*** Nutrients.** Three nutrients, ammonia, nitrate, and orthophosphate, are tested by colorimetric analysis using a LaMotte model MC-1600 colorimeter. Colorimeter specifications are listed on page 4 of the LaMotte notebook of colorimeter test procedures. Nutrient concentrations are determined using percent transmission scales. All water samples are collected and preserved according to requirements for each test (Chapter VII, Table 1).

Assuring accuracy: All colorimeter work is to be completed under laboratory conditions. If the work is done in the field, it is noted on the chemistry data sheet. The colorimeter is set to 100% transmittance for each nutrient with a reagent blank.

Maintenance: Each colorimeter will be calibrated annually with known standards. Any colorimeter found defective will be serviced. Batteries are replaced according to manufacturers recommendations.

*** Ammonia**

Equipment used: LaMotte kit code 3642-WS, a Nesslerization method of detecting ammonia is used with a LaMotte analog colorimeter model MC-1600.

Procedure: Follow LaMotte kit directions.

Assuring accuracy: The colorimeter is calibrated with a reagent blank to account for any reagent color. The reagent mixing waiting period (5 minutes) is timed with a stop watch. Readings are taken directly in front of the needle and recorded as ppm when the needle stabilizes. Three replicates are analyzed. Any replicate that deviates more than +/- 10% of the mean is recorded and reanalyzed.

Maintenance: All reagents are stored and replaced as recommended by the manufacturer. All glassware is washed with distilled water and air dried after each use.

*** Nitrate**

Equipment used: LaMotte kit code 3649-WS, a cadmium reduction method is used with a LaMotte analog colorimeter model MC-1600.

Procedure: Follow LaMotte kit directions. The nitrate test is conducted when the sample water is between 20 and 25 degrees Centigrade.

Assuring accuracy: The colorimeter is calibrated to 100% transmittance with a reagent blank. The reagent mixing waiting period (10 minutes) is timed with a stop watch. Readings are taken directly in front of the needle and recorded as ppm nitrate when the needle stabilizes. Three replicates are analyzed. Any replicate that deviates more than +/- 10% of the mean is recorded and reanalyzed.

Maintenance: All reagents are stored and replaced as recommended by the manufacturer. All glassware is washed with distilled water and air dried after each use.

*** Orthophosphate**
Equipment used: LaMotte kit code 3653-WS, the absorbic acid reduction method for low range orthophosphate, and the LaMotte analog colorimeter model MC-1600. All equipment used to test for orthophosphate including the collection bottle, colorimeter tubes, and any pipets must be acid washed in 5% hydrochloric acid solution for 20 minutes, rinsed three times with distilled water, and air dried.

Procedure: Follow LaMotte kit code 3653-WS instructions.

Assuring accuracy: All glassware is acid washed. Extra care must be taken to prevent contamination of the interior surfaces. The colorimeter is adjusted to 100% transmittance with a reagent blank to account for any interference. The waiting periods for both reagent addition (step 5) and color development (step 6) are timed with a stop watch (5 minutes respectively). Readings are taken from directly in front of the needle and recorded as ppm as soon as the needle stabilizes. Three replicates are analyzed. Any replicate that deviates more than +/- 10% of the mean is recorded and reanalyzed.

Maintenance: All reagents are stored and replaced as recommended by the manufacturer. After each use all glassware is washed with phosphate-free detergent, acid washed in a 5% hydrochloric acid solution for 20 minutes, rinsed three times with distilled water, and air dried. Collection containers are marked as acid-washed and stored with caps on to prevent contamination.

3. Biological Parameters

*** Fecal Coliform**
Equipment used: Millipore (47 mm) aseptic filtration apparatus (model xx1104700); 10 ml graduated cylinders, 1 ml pipets and an incubator supplied by the SEARCH school.

Procedures:
a. Sterilization procedures: All equipment must be properly sterilized according to one of the following methods: autoclaving at 121^0 C and 15 psi for 15 minutes with steam, autoclaving at 170^0 C for 1 hour with dry heat, or boiling vigorously for 10 minutes. Sterile buffer solution is prepared according to the Millipore manual. If sterile buffer is not used, sterile stream water is

made using a 0.22 micrometer filter.

b. Sampling:
i. Field collections: Water is collected in a sterile container from an undisturbed portion of the riffle simultaneously with water chemistry samples. The samples are placed on ice until filtered at the school.

ii. Lab analysis: All work surfaces are washed with alcohol and covered with paper towels. Any person working on the coliform tests washes their hands with disinfectant soap. Forceps are flame sterilized using an alcohol or gas burner before each usage.

iii. *Filtering the sample:* Three sample volumes (1 ml, 10 mls, and 100 mls) are filtered starting with 1 ml. Before the sample is added to the funnel, the collection bottle is lightly shaken to randomly distribute the bacteria. After the sample has been filtered, the funnel is rinsed twice with 20-30 mls of sterile buffer solution. The filter membrane is removed with the sterile forceps and placed in the petri-dish. The filtering apparatus is re-assembled between dilutions in order to prevent contamination. Any time the filtering apparatus is left unused for greater than 30 minutes, or if any part of it comes in contact with any potential contaminant, the entire unit must be re-sterilized.

c. Incubation: The petri-dishes are placed in the incubator for 24 +/- 2 hours at 44.5 +/- 0.2 degrees C. Since incubators are expensive, SEARCH is unable to provide this equipment to schools. Therefore standardization of technique is not possible. It is assumed that there will be large variance in this data, due to variation in the quality of the schools equipment. SEARCH schools are to note incubator type and sensitivity, as well as any deviation from suggested temperatures during the incubation period.

d. Counting: A dissecting microscope is used to count colonies proceeding top to bottom and left to right.

Assuring accuracy:
a. Sterilization. All collection bottles, filtration apparatus, graduated cylinders and pipets must be properly sterilized. Forceps are flame sterilized between every use.

b. Incubation. The incubator is turned on and the temperature maintained at 44.5 degrees C for a minimum of 24 hours prior to field sampling. A water-bath may be installed to reduce any temperature fluctuations.

c. Sample Blanks. Two sample blanks are prepared, one is filtered before field samples and one after all field samples are done. Blanks are run with 100 mls of sterile buffer solution to insure sterile technique.

*** Macroinvertebrates**
Equipment used: All collections are made with a 580 *u*m mesh D-Frame net (Bioquip 7412D).

Procedures:
a. Field collections: Eleven collection stops are completed in areas with adequate flow, starting as close to the left bank (looking upstream) as possible and moving diagonally upstream. A stop consists of placing the net frame securely on the substrate, disturbing one square foot directly in front of the net for a minimum of two minutes or until all of the area has been completely disturbed to a depth of 2-4 inches. The net is removed and contents are emptied into a white bottomed pan. The eleven stops are chosen to represent the different micro-habitats within the riffle. After the final stop, the net is inverted, rinsed, and hand picked until clean. The contents of the pan are run through a 600 um (US #30) brass sieve. The sample is placed in wide-mouthed plastic container, labeled, and preserved with 70% ethyl alcohol.

b. Lab analysis: The sample is placed in a 600 um sieve and rinsed with water.

c. Subsampling: Subsampling occurs in an 18" X 12" X 3.5" polypropylene tray. The bottom of the tray has 28, 2" X 2" squares. Using as little water as possible, the sample is evenly spread across the tray bottom. Square numbers are randomly drawn to determine the sampling order. The contents of a square are removed from the tray. All invertebrates are counted and placed in a separate dish; any debris is discarded. This process continues until 100 organisms have been removed from the tray. If 100 organisms are collected in the first square, the organisms are returned to the tray, the sample redistributed, and the random draw is done again. If 100 organisms are again collected in the first square the sample is adequate. Organisms are not handpicked from squares not randomly drawn. Additional organisms not selected during the random draw may be noted on the Stream Biota Data Sheet.

d. Identification: All identifications are to the family level. Sources used are Merritt and Cummins (1984), Fiske and Byrne (1988), and McCafferty (1981). Additionally, each school has use of a loaner reference collection compiled by SEARCH staff in order to visually confirm identifications. All subsample identifications are confirmed by SEARCH staff. Ten percent of the samples will be selected randomly to be reconfirmed by a State of Connecticut entomologist. To insure future positive identifications, SEARCH schools build their own reference collections. Identification of organisms in these collections is done by a SEARCH staff member.

e. Metric Calculations: Calculations are made according to Plafkin *et al*. (1989) and verified by a SEARCH staff member.

f. Reference storage: Each subsample is placed in an 8 dram vial containing 80% ethyl alcohol, covered with parafilm, and capped with a poly-lined screw top. Labels include site name, county, state, date of collection, and school name.

g. Sample Custody: All subsamples are prepared for storage as described above. Samples are

stored in Hartford at the DEP Office of Environmental Education until donated to a University collection.

Assuring accuracy:
a. Field sampling: Stand to the side of the net while sampling, so as not to disturb the outside of the square foot sample area. Disturb all the large substrate by hand and the smaller gravel by foot for a minimum of 2 minutes per stop. Do not let the net become clogged causing a back up of water. This will cause organisms to float by the net. Do not sample in any area which has already been disturbed. Maintain a tight seal with the stream bottom so that organisms do not slip under the net.

b. Preservation: Use ethyl alcohol whenever possible. Add alcohol until twice the volume of sample material is equaled.

c. Subsampling: Organisms must be preserved; live organisms cannot be scientifically sub-sampled. Once the sample is in the tray it is not disturbed until the subsampling process is complete. All of the organisms from a square are counted even if 100 organisms have been obtained.

d. Identification: SEARCH schools build their own reference collections. The identification of organisms in these collections is verified by a SEARCH staff member.

e. Storage of specimens: All organisms must be kept in ethyl alcohol to prevent decomposition. Organisms are never stored in water for more than 0.5 hours.

C. Internal Quality Assurance/Quality Control Checks

1. Duplicate samples will be collected once in the fall and once in the spring during each school's sampling trip. Water samples are collected simultaneously with the school's collections by SEARCH staff following state protocols. Samples are stored on ice until submitted to the state health lab for analysis. All of the chemical parameters as well as fecal coliform are tested by the state.

2. Ten percent of the duplicate samples will be collected in replicate to monitor state health lab testing.

3. Coinciding with duplicate sample collection during the school trip, a SEARCH staff member will measure dissolved oxygen, stream temperature, and pH.

4. Whenever possible, two schools, which are in the same town or in close proximity to each other, are asked to collect data on a single site. Each school goes to the site independently, no later than one week before or after the companion school.

D. SEARCH Staff Support

1. School Site Visits

a. In the field: SEARCH staff attend as many field trips as possible to provide support and technical assistance to teachers. These visits also increase the credibility of the program to the students, who see "a state scientist" accompanying them. Additionally, SEARCH staff keep a log on anecdotal information detailing class organization, procedures, techniques, areas of difficulty, etc. This information is useful in both evaluating the program and addressing the unique needs of SEARCH schools.

b. In school: Throughout the school year SEARCH staff are available for guest lectures and classroom visits. These lectures help the students on a particular aspect of SEARCH (*e.g.* macroinvertebrate identification, fecal coliform technique, colorimetry, or data analysis) with which their teacher may not feel completely comfortable.

2. Training

a. SEARCH staff: Staff will take workshops and/or classes on subject areas pertinent to the curriculum being taught to the teachers. In addition, staff are encouraged to take research days at university libraries in order to remain current with topical literature.

b. Teacher training: Optional teacher training workshops are offered throughout the school year. These workshops focus on a particular component of the SEARCH program. Several examples are: sterile technique for fecal coliform, macroinvertebrate subsampling, drainage basin mapping, and functional feeding group ecology. Workshops are hosted by either a participating school or SEARCH staff, and held on weekday afternoons.

3. Reference library

SEARCH staff maintain a collection of reference materials pertinent to water quality, wetland, and toxicity studies. Materials include, field guides, taxonomic keys, Standard Methods, journal articles, videos, state water compliance data, and environmental curriculum developed in other states. These materials are available for school usage on an individual basis.

4. Data Analysis

All data sent to the central database, will be reviewed before it is entered. The database will be accessible through the website upon completion of the web database design. This data

will be read only for the general public and can only be altered by the contributing school and the SEARCH coordinator.

A matrix of SEARCH school results versus State of Connecticut Health lab results is maintained to track the evolution of accuracy and precision of each school. Data in the matrix is statistically compared using paired t-tests. These tests allow SEARCH staff to track the progress of each school, as well as identify areas needing additional time and resources.