QUALITY ASSURANCE PLAN FOR
BACTERIOLOGICAL MONITORING
(Addendum to the Quality Assurance Plan
approved on March, 1995)

for

Alabama
Water
Watch

A Program dedicated to developing
Citizen Volunteer Monitoring of
Alabama’s Lakes, Streams and Wetlands
Funded in part by a grant from the U.S. EPA, Region 4
Clean Water Act, Section 319
and the Alabama Department of Environmental Management

prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
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The Alabama Water Watch (AWW) Program is funded in part by a grant from the U.S. Environmental Protection Agency (EPA, Section 319 program under the Clean Water Act) and the Alabama Department of Environmental Management's (ADEM) Office of Education and Outreach. The Department of Fisheries and Allied Aquacultures at Auburn University (AU) is contracted by ADEM to implement, coordinate and provide technical support for the program. A flow chart of the bacteriological monitoring project organization and responsibility charges is presented in Figure 1.

The Program Manager at AU is responsible for overall management (training and technical backstopping of citizen volunteers) and supervision of the technical support staff of the AWW Program. The AWW Program staff develop the AWW Manuals, reports and all information presented at the workshops. The QA/QC Officer is responsible for carrying out the quality control/quality assurance exercises and data management as detailed in the Quality Assurance Project Plan (QAPP) for chemical monitoring (Figure 2), and managing the statewide database. The Monitor Coordinator provides regular contact with citizen groups, conducts training sessions, and coordinates AWW Conferences. Citizen Trainers and Citizen QA/QC Officers assist the Program Coordinators in all aspects of citizen training at workshops and other meetings. The Technical Advisors from a research laboratory in AU’s Department of Fisheries and Allied Aquacultures and Micrology Laboratories provide technical assistance to the AWW Program staff. Qualifications of AWW program personnel involved in the bacteriological monitoring project are presented in Figure 3.

AWW Citizen Volunteers initially attend a six-hour Basic Certification workshop in which they are trained and certified to use water quality test kits to perform specified chemical measurements of water. They are trained in principles of water quality monitoring, proper data collection techniques and data reporting by AWW Program Coordinators and Citizen Trainers. After completing the six-hour Basic Certification workshop, the volunteer monitors are given a certification card and requested to sign a "Liability Release" form (Figure 4).

In addition to the Basic Certification (for chemical parameters), other levels of participation and certification of citizen volunteers are available. Bacteriological monitoring for coliform bacteria (see Appendix A for the AWW Bacteriological Monitoring Manual), and Stream Bioassessment for macroinvertebrates are offered as advanced workshops for volunteers who have been trained in the Basic Certification workshop. Experienced monitors can also complete additional phases of training which qualify them to train new monitors. Training Certifications include: Citizen Trainer for Basic Certification Workshops (chemical parameters), Citizen Quality Assurance/Quality Control Officer (recertification sessions), and Citizen Trainer for Biological Workshops (bacteria and macroinvertebrates).
Figure 1. Bacteriological Monitoring Project Organization and Responsibility Charges

Project Managers
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Public Health

Alabama Water Watch
Citizen Trainers

Alabama Water Watch
Citizen QA/QC Officers

Alabama Water Watch
Citizen Volunteer Groups
and
AWW Association
QUALITY ASSURANCE PLAN

for

Alabama Water Watch

A Program dedicated to developing Citizen Volunteer Monitoring of Alabama's Lakes, Streams and Wetlands Made possible in part by a grant from the U.S. EPA, Region IV Clean Water Act, Section 319 and the Alabama Department of Environmental Management

prepared for

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ADEM Project Director 3-31-95

U.S. EPA Project Manager / QA/QC Officer 3-31-95
Figure 3. Alabama Water Watch Personnel at Auburn University

William Deutsch, Ph.D.
Program Manager (October 1992 - present) Bill has been on the faculty of the AU Department of Fisheries and Allied Aquacultures for 11 years. In addition to AWW responsibilities (six months per year) he works through the International Center for Aquaculture and Aquatic Environments conducting environmental studies and training with projects in the Philippines and Ecuador. He previously worked 11 years with environmental consultants on the Susquehanna River in Pennsylvania.

Wendi Hartup, B.S.
Special Projects Coordinator (September 1997 - present) Wendi has a B.S. in Marine Biology and a Mathematics minor from Troy State University. She has worked at Weeks Bay National Estuarine Research Reserve and Legacy, Inc. revising, editing, and correlating environmental education curricula. Her full-time work with the AWW program involves contact with citizen groups, GIS mapping of sites, conducting training sessions, coordination of the AWW Technical Conference and the AWW/AWWA Annual Meeting/Picnic, serving as the liaison between the AWW Program and Legacy, Inc. and producing reports.

Brooke Smith, B.S.
Monitor Coordinator (April 2002 - present) Brooke has a B.S. in International Business and Management Information Systems. Her work with the AWW program includes updates and maintenance of the AWW website, organization and layout of the AWW waterbody reports, grant reporting, and ETP 16b.

Ron Estridge, M.S.
Data Quality Coordinator (June 1998 - present) Ron worked with AWW on a volunteer basis from June to September 1998. He has an M.S. in Zoology from AU and is retired from the Alabama two-year college system. Ron is also a founding member of Save Our Saugahatchee, Inc., and is currently serving as Chairman of the Board of Directors. His work with AWW primarily involves data entry and processing.

Sergio Ruiz-Córdoa, B.S.
Data Quality Coordinator (April 2001 - present) Sergio has a B.S. in Biology and is a graduate student in the Department of Fisheries and Allied Aquacultures, Auburn University. His work with AWW primarily involves maintaining the statewide database and creating data reports. In addition to AWW responsibilities, he is helping to create a database for the Global Water Watch projects and works with the Ecuador Water Watch group through the International Center for Aquaculture and Aquatic Environments.
Liability

I recognize and understand the potential for injury or loss of property to myself and others under my control or direction arising out of any accident which may result from volunteer activities conducted with Alabama Water Watch's Volunteer Water Monitoring Program. Auburn University, Alabama Water Watch, and Alabama Water Watch Association intends that volunteers expressly assume all risks and liability for any injuries to, or caused by volunteers under this program.

Liability Release

In consideration of the foregoing, I for myself, my heirs, and executors do hereby release and discharge Auburn University, Alabama Water Watch, Alabama Water Watch Association and all supporting organizations for all claim, damages, demands, actions and whatever in any manner arising or growing out of my participation in said monitoring program.

Signed:__________________________ Date: ________________
Several of the the AWW citizen groups have affiliated to form the nonprofit AWW Association (AWWA). The AWWA has a salaried Executive Director, and volunteer President and Board of Directors. Many AWWA members and officers are Citizen Trainers and QA/QC Officers. The AWWA collaborates with the AWW Program for statewide coordination of citizen volunteer efforts.

### Problem Definition / Background

Alabama Water Watch is a network of volunteers from all walks of life who are committed to studying and protecting the lakes and streams of the State. Citizen volunteer monitoring provides baseline information about water conditions to help researchers from educational and government institutions to evaluate and correct water quality problems. Serving as certified water quality monitors is something that people can do to bring about positive change, protecting and restoring our aquatic resource.

**WHY IS BACTERIOLOGICAL MONITORING NEEDED?**
Many citizens are becoming increasingly concerned about potentially harmful bacteria in streams, swimming areas and drinking water. ADEM has only recently begun a pilot beach monitoring program for bacteria, and cases of surface and groundwater contamination by bacteria from faulty septic systems, animal holding facilities wastewater treatment plants and other sources are regularly documented by researchers and governmental agencies in Alabama (Geologic Survey of Alabama, Alabama Department of Public Health, universities, etc.). Bacteriological monitoring is a way for Alabama Water Watch to address these concerns, which adds a human health dimension to the monitors' water testing.

**HOW IS THE INFORMATION USED?**
The process of bacteriological data collection by citizen monitors and use of this data are shown in Figure 5. Volunteer monitors attend an AWW bacteriological workshop and develop sampling strategies depending on their objectives and budget. Monitors regularly send their data to the QA/QC Officer at Auburn University. The information is checked and entered into a computer database by data entry operators for summary and analysis by the Program Coordinators. The AWW Program largely concentrates on data collected over time that may be used to determine long-term trends, and to backstop and advise the monitoring groups on pollution or contamination concerns. Many monitors are able to detect and resolve contamination problems at the local level that need immediate attention. This is often done without regulatory agency involvement or litigation.

The data are presented and disseminated in a variety of formats at Citizen Advisory Council (CAC) meetings with ADEM, through the AWW website and newsletters, at the annual AWW Technical and Public Information Conference and other seminars and through the AWW listserv.
Figure 5. Process of Bacteriological Data Gathering and Use

AWW BACTERIOLOGICAL WORKSHOP

SHARING INFORMATION THROUGH REPORTS, CONFERENCES, SEMINARS, STATEWIDE NEWSLETTERS, EMAIL LISTSERVE, WEBPAGE

CITIZEN MONITORS DEVELOP A CUSTOMIZED SAMPLING STRATEGY

DATA INTERPRETATION SESSIONS

DATA SUBMITTED BY CITIZEN MONITORS AND EVALUATED BY AWW QA/QC OFFICER

GROUP ACTION

"NEIGHBOR-TO-NEIGHBOR" (LOCALLY-LED)

PUBLIC HEALTH DEPARTMENT

ADEM

Alabama Water Watch
6 PROJECT / TASK DESCRIPTION

Coliscan Easygel is the method used to test for *Escherichia coli* (*E. coli*) and general coliform bacteria. This technology was developed by Dr. Jonathon Roth, of Micrology Laboratories, LLC (Appendix C). With this method, a one to five mL sample of water is collected using a sterile, plastic pipette and squirted into a 10 mL bottle of sterile, liquid medium. The general coliforms and *E. coli* each produce enzymes that react with color reagents in the media to produce pink to red colonies (general coliforms) or dark blue to purple colonies (*E. coli*).

AWW monitors perform these bacteria tests on streams, lakes, swimming beaches and wells. Determination of baseline conditions on streams and lakes are the primary reason for bacteria testing. Secondary testing is also done in response to potential contamination problems from broken or leaking septic tanks and sewer lines, waste water treatment plants and animal holding operations. Both point and nonpoint sources of bacterial contamination are detected.

The sampling frequency varies according to the objectives of the citizen groups, and ranges from several times per week to quarterly (many groups sample monthly). Results of the tests are evaluated against EPA Standards (see p. 8 of the AWW Bacteriological Monitoring Manual, Appendix A), with 200-600 *E. coli* colonies/100 mL as the maximum allowable limit for "whole body contact" (depending upon time of year and public use). Monitors are trained in ways to respond to bacteria data indicating contamination levels beyond this recommended EPA limit.

7 DATA QUALITY OBJECTIVES FOR MEASUREMENT DATA

Bacteriological data will only be accepted from monitors who receive training and certification from AWW Certified Bacteriological Trainers.

Data Quality Indicators:

Precision

Precision may be monitored largely through the use of replicate samples. Monitors are trained to collect three replicate samples for each site tested. A high level of precision is generally obtained with replicate samples, though some degree of natural variability is expected when sampling biological parameters.
**Accuracy**

Accuracy of the Coliscan Easygel method is based on the reasonable performance of properly stored, pre-treated sterile plates, media and sterile pipettes. Accuracy of bacteriological plates and media will be determined by periodically comparing multiple measurements of the bacteria test with values obtained from laboratories using APHA Standard Methods (1992). This periodic side-by-side testing will be done by the QA/QC Officer in conjunction with the Fish and Parasite Disease Laboratory at Auburn University's Fisheries Department, Alabama Department of Public Health's County or State Laboratories. An extensive side-by-side study was conducted from February to September 1998 to confirm the accuracy of the technique used by AWW monitors (Figures 6 - 12).

**Reference**


**Measurement Range**

Monitors typically collect a 1 mL sample, but may sample a range of volumes from 0.25 mL to 5 mL. The Coliscan Easygel test can detect as little as one bacterial colony per sample. Citizen monitors are trained to count up to 200 colonies/sample. Higher concentrations are recorded as TNTC (too numerous to count).

**Representativeness**

Representativeness depends on collecting three replicate samples for each site tested at a properly selected site. Monitors receive training in proper site selection that most closely represents the true environmental conditions. For example, monitors are trained to sample appropriate sections of streams above and below potential contamination sources to factor in natural variability and to more closely pinpoint the contamination source.

**Comparability**

Comparability is done through the side-by-side studies of the Coliscan Easygel method with Standard Methods for bacteriological testing. These comparison studies have indicated that the Coliscan Easygel method is a reliable and valid tool for the detection of fecal contamination (Figures 7 -12).

**Completeness**

Once a monitoring group has established a monitoring plan, all bacteriological data are submitted to the AWW office, quality assured, and entered into the statewide database for analysis, summary and dissemination.
Figure 6. Bacteriological Comparison Study Sampling Locations

Bacteriological Study

Alabama Water Watch/ Auburn University Fisheries Department

Saugahatchee and Chewacla Creeks

Tallapoosa River Watershed

Auburn/ Opelika Area, Lee County, Alabama
Figure 7. Number of *E. coli* Colonies/100 mL in Saugahatchee Creek (Site S-1), Lee County, Alabama, from February 5, 1998 through September 1, 1998

# *E. coli* colonies/100 mL

- **Easygel Method**
- **Standard Methods**
Figure 8. Number of E. coli Colonies/100 mL in Saugahatchee Creek (Site S-2), Lee County, Alabama, from February 5, 1998 through September 1, 1998.
Figure 9. Number of *E. coli* Colonies/100 mL in Saugahatchee Creek (Site S-3), Lee County, Alabama, from February 5, 1998 through September 1, 1998

# *E. coli* colonies/100 mL

- Easygel Method
- Standard Methods
Figure 10. Number of E. coli Colonies/100 mL in Town Creek (Site C-1), Lee County, Alabama, from February 5, 1998 through September 1, 1998.
Figure 11. Number of *E. coli* Colonies/100 mL in Parkerson Mill Creek (Site C-2), Lee County, Alabama, from February 5, 1998 through July 28, 1998

# *E. coli* colonies/100 mL

- Easygel Method
- Standard Methods

TNTC

8,700
Figure 12. Number of *E. coli* Colonies/100 mL in tributary to Chewacla Creek (Site C-3), Lee County, Alabama, from February 5, 1998 through July 28, 1998

# *E. coli* colonies/100 mL

- Easygel Method
- Standard Methods
8 Training Requirements / Certification

Volunteer monitors participate in a 2-4 hour training course conducted by the AWW Program Coordinators or AWW Certified Citizen Bacteriological Trainers. The workshop time varies according to the participant's questions and practice time. The bacteriological workshop is considered an advanced workshop for those who have attended an AWW Basic Certification workshop.

The bacteriological workshop objectives include: 1) an introduction to bacteriological testing and water quality standards, 2) demonstration of plate techniques and bacterial counts and 3) sampling strategy and site selection. A local coordinator (interested citizen) usually arranges the logistics for the workshop, which is usually conducted near the monitoring group.

All certified bacteriological monitors are required to attend an annual bacteriological recertification session during which they are updated and evaluated on monitoring and counting of bacterial colonies. The recertification consists of a classroom or lecture period to review techniques and answer questions and practice counts with representative photographs or actual samples that have been plated and incubated to show colonies. Experienced citizen monitors may complete an additional Training of Trainer workshop to become Citizen Bacteriological Trainers. Citizen trainers must also attend an annual Trainer Refresher course.

Most recertifications of bacteriological monitors will be conducted by Citizen QA/QC Officers or the AWW QA/QC Officer at workshops and meetings held throughout the state. To be more responsive to small groups of monitors (five or fewer people), local Certified Bacteriological Trainers may also conduct bacteriological recertification sessions.

9 Documentation and Records

Each monitor must complete a portion of the field data form on-site at the time sampling occurs (Figure 13). This information includes: 1) data collector's name, 2) watershed, 3) monitoring group, 4) date and 5) time the sample was collected, 6) waterbody sampled, 7) AWW Site Code, 8) general site description, 9) general weather conditions, 10) water temperature, and 11) additional observations, 12) the expiration date of the sample media is recorded, 13) the type of sampling method is noted; whether the sample is collected and plated in the field, or collected and transported on ice to be plated indoors and 14) sample volume.

After a 24-48 hours incubation period, the colonies on the entire plate are counted. The number of colonies are recorded on the data reporting form along with the incubation temperature and incubation period.
blue-green colonies may include important genera in the Enterobacteriaceae family (Salmonella, Shigella or others).
• general coliform colonies may include such genera as Klebsiella, Citrobacter and Enterobacter.
• If more than 200 colonies per plate, record as TNTC (too numerous to count).

* Note any evidence of rainfall and runoff within the previous 48 hours.
** This number may be divided by the sample volume and multiplied by 100 for comparison to EPA Standards on p. 8 of the AWW Bacteriological Monitoring Manual.
All original (raw) data are sent to the AWW Program Office and kept on file. Monitors keep a copy of the data for their records. Data are entered and maintained under the supervision of the QA/QC Officer and then are regularly archived on the Auburn University's mainframe computer.

**Sampling Process Design**

There is no experimental design for the bacteriological monitoring, per se. The monitors determine their specific needs and objectives and monitoring strategies are customized to meet these objectives. For example, lake groups may choose to monitor swimming beaches only during the months of June through August, while a stream monitoring group may choose to collect monthly samples year round during base flow for baseline data. Other monitoring groups may suspect a point source pollution problem (for example, a waste water treatment plant) and choose to sample weekly or bimonthly. They are then advised to sample upstream and downstream of the plant.

- **Number of samples required**
  Monitors are trained to collect 3 replicate samples per each sample site and time, so that geometric mean and variability may be determined.

- **Sampling location**
  Whenever possible, monitors on streams should collect samples in water currents that are at least 30 cm/s (about one foot per second) so that measurements are representative of the stream channel. Sampling should be avoided in quiet backwaters or heavily vegetated areas of streams. If a monitor needs to get in the water, the sample should be taken facing upstream without disturbing the sediment.

  Monitors on lakes generally sample swimming beaches, off docks or from boats in open water (e.g. for suspected lake front home septic tank problem).

- **Sampling frequency**
  Sampling frequency is variable, depending on monitoring objectives. Samples should be collected 1-2 times per month for routine monitoring or for screening several new sites, e.g. sampling bacteria at the same time the chemical parameters are being monitored (Quality Assurance Plan for chemical monitoring, Figure 2). Samples should be collected more frequently (1-2 per week) for specific studies of suspected or known contamination problems.

- **Site Selection**
  The general rules for site selection are choosing a site that is: 1) safe, 2) accessible, 3) legal, and 4) strategic, that is, meeting the sampling objectives in an efficient manner. Monitors are required to provide a map or adequate location description for AWW Site Code assignment and GIS geo-referencing. Monitors are also advised to avoid monitoring in dangerous conditions and weather, such as lightning and during flood stage events.
There are several preparatory steps that need to be completed before going to the field to collect samples. The bottles of media should be taken out of the freezer and thawed to room temperature. The sterile Petri dish lid should be taped to the bottom plate on one side to keep the lid closed until the sample/media mixture is added, to prevent contamination. The lid should also be labeled with the date, sampling site, sample volume and replicate number. An incubator (such as a styrofoam cooler) must be set up with a light bulb for a heat source and a thermometer to monitor the temperature. The monitors are advised to test their incubator before incubating bacteriological samples to assure that the optimum temperatures are reached and can be maintained (see the Alabama Water Watch Bacteriological Monitoring Manual in Appendix A).

At each testing site, three replicate samples are collected with a sterile pipette. The same pipette may be used for collecting replicate samples, according to procedure in the manual (see the Alabama Water Watch Bacteriological Monitoring Manual in Appendix A). The medium containing the water sample is poured onto a sterile, pre-treated plastic Petri dish which induces the liquid to solidify. The sample plates are incubated in an insulated styrofoam cooler at 29-37 degrees Celsius (85-99 degrees Farenheit) for 20-48 hours. Following the incubation period, bacterial colonies of \textit{E. coli} and general coliforms are counted and reported.

No sterilizers or glassware are needed for this technique and necessary supplies are easily transported to the sampling sites.

Materials Needed for Each Bacteria Sampling
- 1 mL sterile wrapped plastic pipette for sample collection
- 10 mL plastic Coliscan Easygel bottle of liquid culture medium
- 1 Coliscan Easygel pre-treated Petri dish
- Plate label, pen, tape to secure Petri dish, incubator with heat source, data recording form

Monitors may use one of two methods to collect and plate the samples. The type method used must be noted on the data reporting form.

**Method 1:**
The bottles containing the liquid medium/sample mixture are transported on ice (in a cooler, for example) to the place of plating and incubation (usually at the monitor's house) within 2 hours of the sample collection.

**Method 2:**
The sample is collected and plated in the field. Monitors are instructed on proper handling of the plated samples, such as keeping the Petri dishes level and in the shade as the medium is solidifying.
The sampling supplies are used only once and then discarded after use. Monitors are instructed on proper disinfection and decontamination of the plates before they are discarded (p. 15 of the AWW Bacteriological Monitoring Manual, Appendix A). One teaspoon of bleach is added to each Petri dish and allowed to sit for at least fifteen minutes before disposing in a sealed plastic bag.

**12 Sample Handling and Custody Requirements**

The bacteriological sample remains in the custody of the citizen monitor so that no chain of custody procedures are required. Monitors are trained how to properly label the Petri dishes and bottles prior to doing the field work.

**13 Analytical Methods Requirements**

The Coliscan Easygel method is based on the fact that coliforms produce certain enzymes for lactose fermentation which can be identified (Appendix C). Two chromogenic substrates are acted upon by these two enzymes to produce different color pigments in the Easygel medium. General coliforms produce the enzyme galactosidase and the colonies exhibit a pink to red color in the medium. *E. coli* produce the enzymes galactosidase and glucuronidase and grow purple to dark blue colonies in the medium.

The Coliscan Easygel product is also used under the trademarks "Colichrome" and "Redigel" by 3M Company for use in the food and beverage industry.

**14 Quality Control Requirements**

Preventative maintenance will be done to constantly evaluate common causes of monitoring deficiencies such as broken or faulty equipment; expired, contaminated or insufficient reagents; and inaccurate measuring technique. The training sessions and workshops will emphasize proper handling and maintenance of the equipment and proper testing techniques.

To confirm that the Coliscan Easygel media are not defective, samples will periodically be collected by the QA/QC Officer or AWW Program staff from a site with potentially high fecal contamination. Replicate samples will be taken at each site using 3-5% of the bottles of media, pipettes and plates.
received for distribution to the citizen monitors. These samples will be designated as quality control samples to be analyzed prior to using or distributing the equipment to monitors.

Accuracy of bacteriological plates and media will be determined by periodically comparing multiple measurements of the bacteria test with values obtained from laboratories using APHA Standard Methods (1992). This periodic side-by-side testing will be done by the QA/QC Officer in conjunction with the Fish and Parasite Disease Laboratory at Auburn University's Fisheries Department, or Alabama Department of Public Health's County or State Laboratories.

Monitors are encouraged to collect three samples per site. All acceptable bacteriological data are entered into the computer database. Data with three replicate samples may be graphed more precisely, with averages (geometric mean, Steele and Torrie, 1980) as shown in Figures 7-12 and Appendix B.

Reference

Blue-green or turquoise-colored colonies are reported separately on the data form. These colonies may be important genera in the Enterobacteriaceae family such as *Proteus*, *Salmonella* or *Shigella* or they may indicate defective media which lacks the red indicator. If an excessive number of blue-green colonies are detected, monitors are encouraged to resample (preferably, with a different batch of media). If high numbers of blue-green colonies are still evident, the program office will try to confirm results or send media from a different batch for retesting.

If *E. coli* colony concentrations are detected that exceed EPA standards, monitors are instructed to make repeat measurements for verification. Cross-checks may need to be made by a different field team or monitor, if possible. If the problem is verified by AWW monitors, a professional assessment is requested by an appropriate agency such as ADEM or the Health Department.

The QA/QC Officer will review the data sheets to identify potential problems such as incomplete or incorrect information, expired sample media, etc. and will only enter data that has been received from water monitors who have been certified in an AWW Bacteriological Workshop. Monitors will be required to attend an annual recertification session to review technique.
Instrument / Equipment Testing, Inspection and Maintenance Requirements

All bacteria sampling supplies and equipment used by citizen monitors in the AWW Program are purchased from Micrology Laboratories. Upon receipt of the supplies, the bottles of media are immediately placed in a freezer or refrigerator for storage until use. The recommended shelf-life of the media is six months in the freezer or two weeks in the refrigerator. Expiration dates for the frozen media are printed on the bottles by Micrology Laboratories. Monitors are advised to write this expiration date in an obvious location on the box containing the bottles of media to emphasize the importance of using the materials within the recommended time period.

The packaged pre-treated Petri dishes and pipettes are inspected for defects (i.e. broken dishes and opened pipette packages) and discarded if found to be defective.

The workshop training and manual provide instructions to avoid contamination before sampling, such as keeping the sterilized pipette in the wrapper until sampling and taping the Petri dishes closed for transport to the field.

Instrument Calibration and Frequency

No calibrations are performed on the equipment and supplies used for bacteriological monitoring. All supplies and equipment are used only once, then discarded (procedures for disposal of equipment are discussed in the AWW Bacteriological Monitoring Manual, p. 15, Appendix A).

Inspection and Acceptance Requirements for Supplies

Supplies are inspected when they are received from Micrology Laboratories. Broken or defective supplies are returned for replacement. The AWW Program office or citizen monitors must have a functional freezer to store orders of bacteriological media.
DATA ACQUISITION REQUIREMENTS

U.S. EPA Standards for \textit{E. coli} in water, based on 1986 EPA report, are used by monitors to compare their results. In addition, Dr. Jonathon Roth, inventor of the Coliscan Easygel product and a microbiology professor, is consulted by the AWW Program office for technical advice on the use of this product and in bacteriological monitoring in general.

In certification workshops, bacteriological monitors are encouraged to use local maps and other information to identify potential sources of bacteriological contamination, such as location of waste water treatment plants, animal holding facilities, etc.

A Stream Walk guide, for recording watershed and stream characteristics, is provided to all trainees during the AWW Bacteriological Workshop. This workshop is a prerequisite for AWW Bacteriological Training.

DATA MANAGEMENT

Field data will be recorded on a carbonless, triplicate data reporting forms as shown in Figure 13. This data form will be mailed to the AWW Program Office at AU's Department of Fisheries and Allied Aquacultures after each sampling. Another copy is retained by the monitor and the third copy is filed with the monitor's group.

The data reporting forms will be "logged in" the order they are received by data entry personnel. A record of the log-in will be maintained to document data flow and data checks.

The data will be checked as follows:

1. The data reporting forms will first be screened by the QA/QC Officer for errors or problems such as missing data, dates, times, incorrect units, illegible handwriting, improper decimal placement, or obvious outliers. Most of these errors will be able to be resolved by contacting the volunteer monitors by phone or mail. If extreme readings are found that cannot be obviously explained (for example, high contamination from sampling after heavy rain event or below a waste water treatment plant), the monitor will be requested to test again or to contact another monitor to resample, in order to rule out inaccurate testing technique or faulty media.

2. The QA/QC Officer will mark that the data sheets have been checked prior to data entry. All data will be entered into a spreadsheet-type application program designed for this project and compatible with software used by the state and other water resource agencies.
3. Each data reporting form is assigned a unique reference number which indicates year and order in which received (e.g. 99-001, 99-002, etc.).

4. The data entered into the computer will be printed and checked against the original data reporting forms and corrections made.

5. A second check will be done of the original data reporting forms against a printout of the corrected data.

6. The data will then be marked as ready for analysis.

7. In addition to original data being maintained in the AWW office, data are regularly backed up and archived onto AU’s mainframe computer.

**Assessments and Response Actions**

The review of monitor's activities and data is the responsibility of the AWW Program Coordinators and QA/QC Officer. Volunteer citizen monitors will receive annual bacteriological recertification training.

All bacteriological data is collected by certified AWW monitors. Monitors are recertified annually or biannually, at the discretion of the Citizen QA/QC Officers, and are provided with regular technical backstopping by the AWW Program personnel, AWW Citizen Trainers and Citizen QA/QC Officers.

A toll-free telephone number and email address for the AWW office is provided to all monitors to facilitate communication regarding techniques, water quality problems, or other concerns.

**Reports**

All bacteriological data will be presented in Semi-annual and Annual reports, prepared by the AWW Program Coordinators and submitted to ADEM (Office of Education and Outreach, Chief of Water Division, Chief of Field Operations Division, Director and Deputy Director), AU’s Contracts and Grants Office and the AU Fisheries Department Head. These reports consist of overall assessment of the program’s activities, which include QA/QC protocol implementation, results of QC activities from the recertification workshops, replacement of reagents and equipment, number of data reporting
forms received, and status of the data. The reports also summarize the number of bacteriological monitoring sites and samples by group, watershed and county.

**Data Review, Validation and Verification Requirements**

All AWW field data will be reviewed by the QA/QC Officer to determine if the data meet the QAPP objectives based on the criteria for precision, accuracy, representativeness, comparability and completeness.

Decisions to reject data are made by the QA/QC Officer, but may require additional review for validity by the Program Manager and/or Monitor Coordinator. Acceptance of data is based on the verification that the monitor is certified, that sample media is fresh and that the site is representative.

**Validation and Verification Methods**

Every opportunity will be made to assure data quality. Reviews of testing techniques, data collection and common mistakes made will be highlighted at workshops, meetings and at field audits throughout the year.

The field data sheet has a place for observations that would include a notation for rainfall events in the 48 hours prior to sampling, in order to account for high bacterial concentrations that may be associated with runoff.

Nonsensical data is difficult to determine because bacterial concentrations are often not predictable. High variability among replicate samples is cause to suspect sampling error, and monitors are encouraged to verify such results with additional tests.

Formulas for calculations (# colonies/volume sampled) are incorporated in the database to ensure accurate and standardized results.

Besides the efforts made at workshops and training sessions to control data quality, additional steps will be taken such as training the monitors on how to check their own data before sending it to AU, and the importance of carefully completing all sections of the data reporting forms.
Monitors are trained in the bacteriological workshop to properly identify the types of colonies present based on their color and to calculate levels of *E. coli* and general coliform colonies per 100 mL. They are also trained to verify high levels of *E. coli* with additional tests, or to contact the AWW Program office, their local public health department or ADEM for an official assessment.

Laboratory studies indicate that the techniques we use usually result in low variability of *E. coli* concentration, therefore monitors are instructed that high variability among replications are reason to resample to verify their initial data.

The bacteriological data collected by citizen monitors are not used for enforcement or regulation and are considered to be a "first alert." Problems detected and verified by the citizen monitors will receive further attention by professionals.
Appendix A

Alabama Water Watch
Bacteriological Monitoring Manual
Bacteriological Monitoring Manual

Certification Workshop Objectives:

• Introduction to Bacteriological Testing and Water Quality Standards

• Demonstration of Coliscan Easygel Sampling Techniques

• Practice Sampling Techniques and Counting Colonies on Plates

• Discussion, Questions, Comments, Monitoring Plans

The Alabama Water Watch Program fosters the development of Citizen Volunteer, Water Quality Monitoring Groups through training, technical advice, group backstopping and dissemination of citizen data statewide. This project is partially funded by the Alabama Department of Environmental Management through a Clean Water Act Section 319 (h) nonpoint source grant provided by the U.S. Environmental Protection Agency, Region IV.

For more information, call the Alabama Water Watch Program office at the Auburn University Department of Fisheries: 1-888-844-4785 (toll free).

email: aww@acesag.auburn.edu
http://www.auburn.edu/aww
**Introduction to Bacteria Testing**

Levels of potentially harmful bacteria in streams, swimming areas, wells and piped drinking water are becoming an increasing concern to many citizens. In 1996, Alabama Water Watch began testing for bacteria in addition to other parameters used for water quality assessment because of obvious health risks and relevancy to citizen monitors.

Sources of bacteria in water:

- septic tanks
- waste water treatment plants
- broken or leaking sewer lines
- sewer overflow (especially during rainfall events)
- animal feeding operations
- wildlife

Bacterial contamination from human and animal sources is the most commonly detected drinking water problem nationally and in Alabama. Animal waste pollution is becoming an increasing problem in the U.S., and such contamination has been documented in surface and ground water, resulting in unsafe swimming areas and wells.

**Coliform bacteria** are members of the *Enterobacteriaceae* family. Several species of bacteria in this family are normally found in soil and water and are not pathogenic. Technically, coliforms are defined as gram negative, anaerobic, non-spore forming rods which ferment the sugar lactose with the production of gas and acids. Some of the coliform bacteria live in the intestines of warm-blooded animals (birds and mammals), and are called **fecal coliforms**.

*Escherichia coli* (*E. coli*) is a type of fecal coliform, and exists as some 700 strains (most of these strains are harmless). *E. coli* is the primary bacterium in the intestinal tract of warm-blooded animals (and thus their stools), and its presence in food or water indicates fecal contamination. This is why *E. coli* serves as a sentinel or **indicator organism**, indicating the need for intervention due to fecal contamination.

*E. coli* contamination suggests that other, potentially dangerous bacteria, such as *Salmonella* and *Shigella* (which are important non-coliform types), could also be present.
Traditional tests for coliforms and fecal coliforms require a fairly involved process using specialized media, a series of dilutions with buffers, inoculation, and incubation under carefully controlled temperatures. This approach is expensive and time-consuming, requiring specialized laboratory equipment and a specially trained person to perform the test.

Coliscan Easygel, a relatively new technology developed by Dr. Jonathon Roth, of Micrology Laboratories, offers several advantages over the traditional Standard Methods for bacteria testing. It is simple to use, inexpensive, and an accurate and quantitative way to identify and differentiate *E. coli* and general coliforms in water.

These advantages make the Coliscan Easygel method an excellent “first alert” tool. This is particularly useful for natural resource managers, which could be followed with an additional assessment, such as from the Public Health Department or a certified laboratory.

The Alabama Water Watch Program has worked closely with Dr. Roth to develop bacteriological sampling protocols for citizen monitors. In addition, testing results, questions and comments from AWW monitors and trainers have led to the continual refinement and improvement of protocols and training materials.
E. coli traced to water park

At least 12 children sick from bacteria outbreak

By Patricia Guthrie
STAFF WRITER

A popular kiddie pool area at Cobb County's White Water park is the source of an E. coli bacteria outbreak that has sickened at least a dozen children in three Southeastern states, Georgia health officials said Tuesday.

"We have a 98 percent certainty children defecating in the water resulted in an acute contamination," Virginia Galvin, director of Cobb County Public Health department, said at a press conference.

The contamination occurred June 11 and 12 in the White Water wading pools in an area called "Captain Kid's Cove," officials said. Authorities said it is unclear which specific pool spread the contamination and which child, or children, triggered the outbreak.

Three of the children also attended the same day care facility run out of a private home in Bartow County but they were all taken to play at White Water on those particular dates, Galvin said.

White Water spokeswoman Deedie Dowdle said it is unclear where the contamination occurred.

"There's no way we could know where the kids played when they were here," she said. "We do..."
WORLD VIEW

WATER: NOT A DROP TO DRINK

Nearly a billion people in 50 countries live with severe shortages. What can the world do?

BY PRANAY GUPTE

A gathering crisis: Fetching water in Haiti

to the edge of extinction from contaminated water.

Unfortunately, such statistics don’t seem to be persuasive enough for world leaders to act expeditiously, or meaningfully, on water-management issues. “Everyone lives downstream,” was last week’s catchy slogan marking World Water Day, but few in the tightly knit world of development aid actually do much about the state of the stream itself in poor nations.

The glaring lack of attention to water issues seems especially puzzling in light of the fact that the estimated cost to provide safe water in rural areas is $50 per person per year and about $100 per person in cities, according to U.N. estimates. In a report released last week, the United Nations estimates the overall price to bring low-cost safe water and sanitation to all those who need it at around $25 billion annually over the next decade. Current world investment in water-related development projects is $8 billion per year, or a shortfall of $17 billion—an amount roughly equal to annual pet food purchases in Europe and the United States, notes Toepfer.

The hapless Swedish developmentalist who neglected to ascertain whether there was indeed water available in his African village may not have been entirely naive. Developing countries do indeed need low-cost technologies such as hand pumps, gravity-fed rainwater collection systems. But these devices can hardly work effectively unless aid agencies coordinate their efforts better (the Swede had neglected to consult local hydrologists). Sophisticated indoor plumbing may not be practical for existing hovels in poverty-stricken neighborhoods; resources could be more effectively channeled into building new homes for growing populations. That is why, as development mandarins fashion their strategies for the new millennium, water-management issues must be considered in tandem with housing, health and social development.

As much of the developing world becomes urbanized, its water crisis will deepen. Large cities already bursting at the seams—Mexico City, Lagos, Dhaka and Cairo—rely largely on ground water, but aquifers take decades to recharge while the population growth in such cities is exponential. By next year, 20 cities in the developing world will have populations exceeding 10 million. And as urban demands for water increase, supply for the developing world’s already water-starved agricultural areas will be further affected, thereby creating a potentially monumental food-security crisis.

All of this suggests that in an increasingly globalized world, a more coherent strategy for economic and social development is urgently needed. Hydrologists say that the world’s water supply is finite—less than a million cubic kilometers that, according to the United Nations, is not sufficient for today’s global population, which is growing at the unsustainable rate of 100 million people annually. UNEP’s Toepfer wasn’t engaging in hyperbole last week when he told Newsweek: “My fear is that we’re heading for a period of water wars between nations. Can we afford that in a world of globalization and tribalization where conflicts over natural resources and the numbers of environmental refugees are already growing?” Chilling words, scary scenario, terrifying prospect.

Gupte is editor and publisher of The Earth Times

NEWSWEEK MARCH 29, 1999
Main Genera in the *Enterobacteriaceae* Family of Bacteria

**Enterobacteriaceae Family**

- **NON-COLIFORMS**
  - *Shigella*
  - *Salmonella*
  - *Yersinia*
  - *Providencia*
  - *Serratia*
  - *Proteus*

- **COLIFORMS**
  - **GENERAL**
    - *Escherichia*
    - *Citrobacter*
    - *Klebsiella*
    - *Enterobacter*
  - **Fecal**
    - *Escherichia coli*
    - *Citrobacter freundii* (*
    - *Klebsiella pneumoniae* (*

* Some strains of these may be of fecal origin, but most are not
U.S. EPA Standards for *Escherichia coli* in Water

Single sample, maximum allowable, *E. coli* colonies per 100 mL of water (based on 1986 Report: Ambient Water Quality Criteria for Bacteria, U.S. Environmental Protection Agency, Washington, D.C., EPA 440/5-84-002)

Piped Drinking Water 0

Drinking Water Source (pre-treatment) 2,000-4,000

Designated beach area 235

Moderate swimming area 298

Light swimming area 406

Rarely used swimming area 576

The *E. coli* concentrations that are safe for “whole body contact” are the equivalent of about 2 to 6 colonies of *E. coli* per 1 mL sample of water, depending on the use (frequency) of the water body for swimming. These standards are used for a waterbody that is use-classified as “swimmable-fishable.”

The values above, related to “whole body contact” standards apply to summer months when swimming is likely. Permissible concentrations of *E. coli* are higher in the winter months, when whole body contact is less likely, and generally during initial runoff from rainfall events (“first flush”).

These are standards for collecting multiple samples in a short period of time (for instance, samples collected to check contamination at a public beach). If five or more samples are collected in a 30-day period (no two samples spaced less than 48 hours apart), the geometric mean of the samples should not exceed 126 *E. coli* colonies per 100 mL for waters classified as swimmable (U.S. EPA, 1986).
Before Going to the Field to Collect Your Samples

1. Prepare the media:

   • Take the bottles of media out of the freezer before sampling (or the day before sampling) to be sure they have reached ambient temperature.

   Write the date you receive the media and the expiration date on the box of media when you receive it. If you purchase supplies for more than one sampling date, the bottles of media should be kept frozen until use. Media may be frozen for up to six months and still be usable. Thawed medium is usable for up to two weeks. Medium may be refrozen and rethawed, but this should be avoided. Discard any expired media. Use of expired media may result in inaccurate readings.

2. Prepare the Petri dishes:

   • Tape the Petri dish lid to the bottom with two pieces of peelable tape (opposite sides of dish, forming a “latch” and “hinge”). Keep the Petri dishes closed until you add the sample/media mixture to prevent contamination.

   • Label the plate lids with indelible ink (write on tape or use a stick-on label). Label should include date, sampling site, sample volume and replicate number. Usually, three replicate samples are collected at each site (replicate sample: two or more samples collected at the same place and time, used to measure variation in bacterial counts).

3. Set up an incubator:

   • The plates should be cultured at 29-37 degrees C (85-99 degrees F). This may be done in a simple incubator, made with a Styrofoam cooler or styrofoam bait box etc. with a night-light bulb for a heat source. Alternatively, plates may be incubated on top of a freezer or refrigerator or other warm surface (if the culture temperature is reached).

   • The thermometer from the AWW water chemistry test kit, or a dial thermometer (found in garden centers or Feed and Seed stores and inserted through the incubator lid) may be used to monitor the incubator temperature.
- Avoid placing the cultured plates in direct sunlight or other source of ultraviolet (uv) light (this may kill bacterial cells).

- The incubator should be tested before sampling to assure optimum incubation temperatures are reached and can be maintained.
Field Techniques for Bacteriological Sampling

1. Leave the pipette in the sterile wrapper until ready to take the sample. Unwrap the pipette from the bulb end and avoid contacting the tip of the pipette with anything except the sample water, being careful not to collect sediment from the bottom of the stream or lake.

2. Squeeze the pipette bulb, insert tip into the water to be sampled (about 2-3 inches deep) and release the bulb slowly, drawing up the sample into the pipette. Squeeze out excess sample water to the 1 mL line.
   
   • Do not insert your hand into the water.

   • Collect your sample upstream of where you are standing if you need to get into the stream.

   • You can collect up to 5 mL of water per bottle, but 1 mL is usually sufficient. For most surface waters, a 1 mL sample will reveal as low as 1 \( E. coli \)/mL (100 \( E. coli \)/100 mL sample).
     A 5 mL sample will detect as low as 1 \( E. coli \)/5 mL (20 \( E. coli \)/100 mL sample).

   • If water is known to be contaminated, sample volume may be reduced to 0.75, or 0.5 or 0.25 mL to make the counting of colonies easier.

   • It is very important to record the sample volume on the dish label and data sheet.

3. Squirt the sample water into the bottle of liquid Coliscan Easygel medium without placing your hand over the open bottle. The pipette and bottle may be angled to ensure that the sample water is delivered into the bottle and not spilled. Cap and gently swirl the bottle to mix the sample water and medium.

   • We recommend three samples be collected from the same location using three different bottles of media. These will be your replicate samples 1, 2 and 3 on the data reporting form.

   • One pipette may be used to collect all three replicates as long as you are careful not to touch the tip to anything except the sample water.
4. Two methods may be used to collect and plate the samples (Method 1 is preferable).

**Method 1.** After sample collection, place the labeled sample/medium bottles on ice and plate indoors within 2 hours of collection.

- Bottles may be put in a plastic bag (zip-lock baggie) and placed on ice in a small cooler or other container.
- Saves time, especially if more than one sample site is being tested.
- No need to wait or risk disturbing the poured medium before it completely hardens.
- Multiple samples may be poured into dishes on a level surface indoors, and completely solidify without disturbance.
- Important to label bottles so that they may be associated with the labeled cultured plates.

**Method 2.** Collecting and plating the samples in the field.

- Useful if collecting bacteria and water chemistry data at a single site.
- Use a covered box to keep the plated samples out of direct sunlight and to transport.
- Streak or “spreader” colonies may result if sample/medium mixture is not completely solidified before transporting.
After Sample Collection, Plating the Samples

1. Peel back the “latch” piece of tape on the Petri dish and lift the lid just enough to pour the contents of the bottle into the plate. Do not splash the medium onto the sides or lid of the plate.

2. Tape the plate shut and gently swirl it in a circular motion to evenly disburse the medium across the bottom of the Petri dish.

3. Place the plate on a level surface (out of direct sunlight) and allow the medium to harden (gel) for about 30 minutes to one hour.

4. Invert the plate and place in an incubator or warm, dark place to culture it “upside down” (this will prevent condensation water from dripping onto the plate from the lid). Incubation usually takes about 24-48 hours at 29-37 degrees C (85-99 degrees F). Plates should not be incubated for more than 48 hours.

   • Normal room temperatures will usually be below the recommended incubation temperature range.

   • The higher temperature range is better because it favors the growth of members of the Enterobacteriaceae family to which the general coliforms and E. coli belong. The Coliscan Easygel medium contains inhibitors to prevent many other bacteria from growing.
### Determining the Results

1. Count the **entire** plate.

   - *E. coli* colonies will range in color from dark blue to purple; general coliforms will range in color from pink to dark red.

   - Bright, blue-green or turquoise colonies may be related to the **Enterobacteriaceae**, but are probably not coliforms. This non-coliform group can contain members of the genera *Salmonella* and *Shigella* which can be pathogenic. Count these colonies and record data separately on the reporting form.

   - Only count colonies that are readily visible to the naked eye. Do not use a hand-lens to count colonies and do not count “pin point” sized colonies (<0.3 - 0.5 mm).

   - If the number of colonies of a particular type (*E. coli* or general coliforms) exceeds 200 per plate, report it as TNTC (too numerous to count).

2. Divide the number of *E. coli* colonies on the plate by the sample volume and multiply by 100 to estimate *E. coli* bacteria per 100 mL. Compare this value to the EPA standards.

**Example calculations:**

<table>
<thead>
<tr>
<th># E. coli colonies/plate</th>
<th>Sample Volume (mL)</th>
<th># E. coli colonies/1 mL</th>
<th># E. coli colonies/100 mL</th>
<th># General coliform colonies/plate</th>
<th># General coliform colonies/100 mL</th>
</tr>
</thead>
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<tr>
<td>5</td>
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<tr>
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<td>0.5</td>
<td>10</td>
<td>1000</td>
<td>20</td>
<td>4000</td>
</tr>
</tbody>
</table>
Decontamination, Plate Disposal and Incubator Maintenance

1. Avoid contaminating hands, table tops, etc.
   - Do not touch bacterial colonies.
   - The dishes should be taped shut and kept out of reach of inquisitive children or pets.
   - Keep a disinfectant nearby to clean table tops or other areas that colonized plates have touched (especially if liquid spills from plates).
   - Always thoroughly wash your hands after handling the plates.

2. An easy and effective way to decontaminate the contents of the Petri dish is to lift the lid and pour about a teaspoon of (undiluted) Clorox or equivalent bleach into each dish. Retape the lid, gently swirl the plate to distribute the bleach, and allow the dish to stand for about 15 minutes to kill the microbes.

3. Dispose of the decontaminated Petri dish in a sealed plastic bag.

4. Periodically wipe or spray the inside of your incubator with dilute bleach solution or Lysol spray and let air-dry before next use.
Reliability and Applications of Coliscan Easygel Techniques

Studies were conducted by AWW and the Parasite and Disease Lab in the Department of Fisheries and Allied Aquacultures at Auburn University to compare the Coliscan Easygel Method with Standard Methods. Replicate samples were collected at six different sites on two waterbodies in the early Spring and mid- to late summer in 1997 and 1998. Results indicated that the Coliscan Easygel method is a reliable and valid tool for the detection of fecal contamination through a wide variety of concentrations (see graph).

The Coliscan Easygel method was used to analyze both drinking water and surface water in the rural Philippines, as part of a collaborative research project. The results were consistent for a given watershed across seasons, and correlated with patterns of soil erosion, land use, human population and overall environmental degradation (see graph).
Number of *E. coli* Colonies/100 mL in Saugahatchee Creek (Site S-2), Lee County, Alabama, from February 5, 1998 through September 1, 1998

# *E. coli* colonies/100 mL

![Graph showing the number of *E. coli* colonies per 100 mL over time from February 5, 1998 to September 1, 1998, with two methods indicated: Easygel Method and Standard Methods.](image)
Total suspended solids, land use patterns and concentrations of *E. coli* bacteria in four subwatersheds of the Manupali River, August 1995 - July 1996

(AU Study using Coliscan Easygel)
How to Get Started with Bacteria Monitoring in Your Area

• Attend an AWW Bacteriological Monitoring Workshop and become certified
• Make a plan for sites to monitor, with clear objectives
• Purchase necessary supplies
• Use careful technique for accurate results
• Submit data regularly to AWW Program office
• Plan data interpretation sessions with AWW Program office

What if Fecal Contamination is Found?

If you suspect that your sampling site is dangerously contaminated based on the results you get from your testing, you may need to test the site again. You may also want to verify this with an AWW trainer or the AWW Office first. Try to find the potential source of contamination. If the problem is verified, you should contact your local health department or ADEM and ask for an official assessment of the water.

Materials Needed for Each Bacteria Sample (Coliscan Easygel from Micrology Laboratories):

• 1-mL sterile wrapped pipette
• Plastic bottle of liquid medium
• Pre-treated Petri dish
Coliscan Easygel Ordering Information
Micrology Laboratories
P.O. Box 340
Goshen, Indiana 46527
PHONE: 1-888-EASYGEL (toll-free)
FAX: 219-533-3370
EMAIL: micrologylabs@juno.com

• Coliscan Easygel (Cat. no. 25001) - $1.75 each, $1.25 for 100 box, (includes individual 12.5 mL bottles of medium and pre-treated Petri dishes)

• 1 mL sterile pipettes (cat. no. DRP01) - $0.12 each

• check with Micrology Labs for current prices
**SAMPLING SITE FORM**

* Be sure to send a map which highlights where your site is located in relation to county roads or other key landmarks.

Name _____________________________________________________________

Phone Number ____________________________________________________

Group Affiliation __________________________________________________

Waterbody _________________________________________________________

County and State Where Site Is Located ________________________________

**Site Description:** (Be very detailed. Include information such as whether or not it is near a road crossing, upstream or downstream of a bridge, etc.)

______________________________________________________________________________

______________________________________________________________________________

______________________________________________________________________________

Did you take a picture of the site?  □ Yes  □ No

Group Site Code Number (if applies) _________________________________

______________________________________________________________________________

**DO NOT WRITE BELOW THIS LINE. AWW OFFICE USE ONLY**

AWW Site Code Number* ____________________________________________

* An 8-digit number assigned by the Water Watch Office.
Bacteriological Quality Assurance Plan

### Alabama Water Watch

<table>
<thead>
<tr>
<th>Collector</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Watershed</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Date collected (m/d/y)</td>
<td></td>
</tr>
<tr>
<td>Time of collection</td>
<td></td>
</tr>
<tr>
<td>Waterbody</td>
<td></td>
</tr>
<tr>
<td>AWW Site Code</td>
<td></td>
</tr>
<tr>
<td>General Site Description</td>
<td></td>
</tr>
<tr>
<td>General Weather Conditions</td>
<td></td>
</tr>
<tr>
<td>Water Temperature (°C)</td>
<td></td>
</tr>
<tr>
<td>Incubation Temperature (°C)</td>
<td></td>
</tr>
<tr>
<td>Incubation Period (hours)</td>
<td></td>
</tr>
<tr>
<td>Observations*</td>
<td></td>
</tr>
</tbody>
</table>

Expiration date of sample media _____________

Sampling method (check one):

- [ ] plated at site, or
- [ ] sample collected in media bottle and transported on ice before plating (2 hours or less)

<table>
<thead>
<tr>
<th>Replicate #</th>
<th>Sample Volume (mL)</th>
<th># E. coli colonies/plate** (dark blue-purple)</th>
<th># General coliform colonies/plate (pink-dark red)</th>
<th># Blue-green colonies/plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- blue-green colonies may include important genera in the Enterobacteriaceae family (*Salmonella*, *Shigella* or others).
- general coliform colonies may include such genera as *Klebsiella*, *Citrobacter* and *Enterobacter*.
- If more than 200 colonies per plate, record as TNTC (too numerous to count).

* Note any evidence of rainfall and runoff within the previous 48 hours.

** This number may be divided by the sample volume and multiplied by 100 for comparison to EPA Standards on p. 8 of the AWW Bacteriological Monitoring Manual.
Appendix B

Tabulated Comparison of Coliscan Easygel with Standard Methods Data
### Bacteriological Comparison Data of Coliscan Easygel Method vs Standard Methods from February 5, 1998 to September 1, 1998,
Alabama Water Watch Program, Auburn University

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### Bacteriological Comparison Data of Coliscan Easygel Method vs Standard Methods
from February 5, 1998 to September 1, 1998,
Alabama Water Watch Program, Auburn University

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from February 5, 1998 to September 1, 1998,
Alabama Water Watch Program, Auburn University

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Appendix C

Principles and Techniques for Using Coliscan Easygel
(Micrology Laboratories, LLC.)