IOWA'S WATER
Ambient Monitoring Program

Citizens Monitoring Bacteria

Background

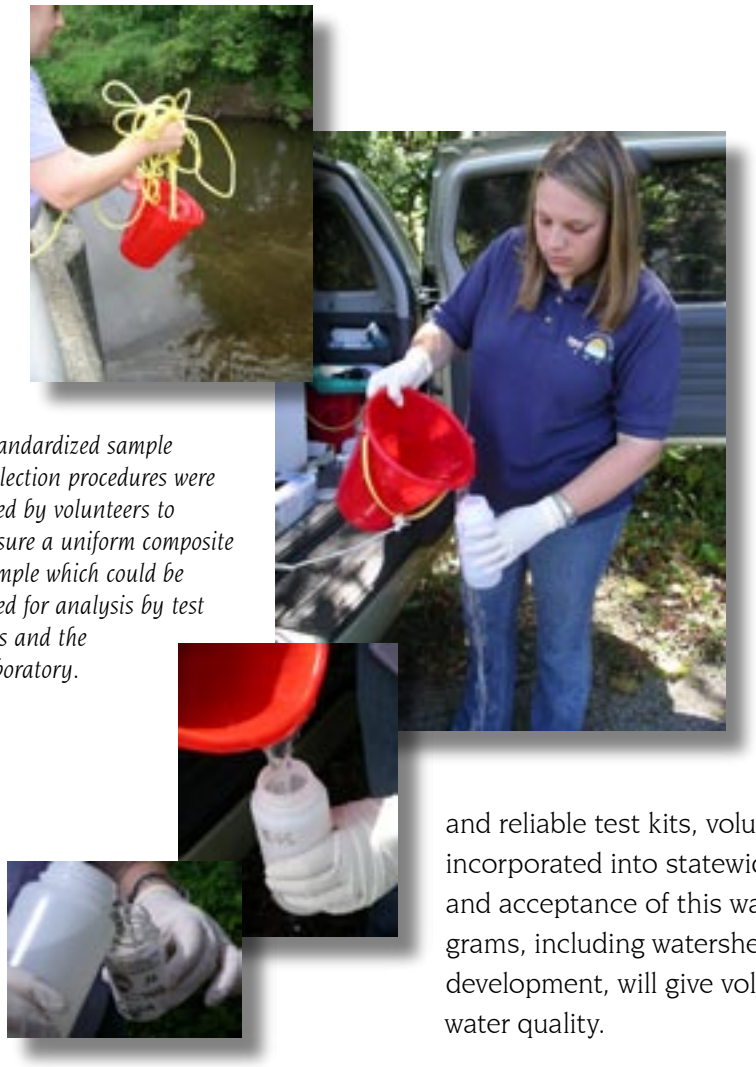
Bacterial contamination of surface waters is a priority issue in watershed restoration for many states in the Upper Midwest. However, state and local agency resources may not be adequate for the needed sample collection and analysis costs. Developing a network of trained volunteers capable of producing accurate and reliable results is the goal for the three-year project headed by a team of university and agency partners from Indiana, Iowa, Michigan, Minnesota, Ohio, and Wisconsin. The Midwest Bacteria Project, a United States Department of Agriculture funded project, will evaluate bacteria test kits, create a comprehensive training program, and develop public outreach materials.

In 2004, trained volunteers from Iowa's IOWATER Program and Indiana's Hoosier Riverwatch collected stream grab samples in order to evaluate the accuracy, reliability, and usability of several commercially available *E.coli* test kits. Test kits chosen for this project included Coliscan® Easy Gel (incubated and not incubated), Coliscan® Membrane Filtration, 3M™ Petrifilm™, and IDEXX Colisure™. Each test kit has known advantages and disadvantages for use by volunteers. One objective of this study was to evaluate each kit's performance based on numerous criteria including accuracy, ease of use, cost, and volunteer input. At the end of the study, researchers will determine the best test kit(s) to be used by volunteers who wish to test for *E.coli* bacteria.

The Study

For the first year of this study, standardized field and laboratory procedures were created to ensure reliability of results provided by volunteers. Samples analyzed by the volunteers were compared to split samples sent to a single laboratory (University of Iowa Hygienic Laboratory) certified by the United States Environmental Protection Agency in *E.coli* analysis. Split samples were taken from a larger composite bottle to ensure uniformly mixed samples. Throughout the first year, volunteers were able to compare results obtained by the lab to those determined from their test kits.

Following the sampling season, volunteers from Iowa and Indiana were surveyed to determine the usability of the various test kits. Based on survey results, along with comparisons between lab and volunteer data, the project team is working to identify and recommend the test kit method(s) that best combines accuracy, ease of use, and cost.



Standardized sample collection procedures were used by volunteers to ensure a uniform composite sample which could be used for analysis by test kits and the laboratory.

After a test kit has been recommended, new volunteers from all states involved in the Midwest Bacteria Project will be trained in 2005 with consistent training methods. Questionnaires given at each training session will establish the basis of volunteer knowledge in current water quality and bacterial contamination issues. Volunteer knowledge and proficiency in the use of the chosen test kit will then be tracked over time. Based on these results, the training curriculum will be assessed and revised as necessary to produce volunteers proficient in *E.coli* bacteria monitoring.

Through the use of standardized sampling and analysis procedures as well as accepted and reliable test kits, volunteer collected *E.coli* data can be useful and incorporated into statewide water quality data. Increased awareness and acceptance of this water quality data in various watershed programs, including watershed assessments and Total Maximum Daily Load development, will give volunteers an increased stake in their state's water quality.

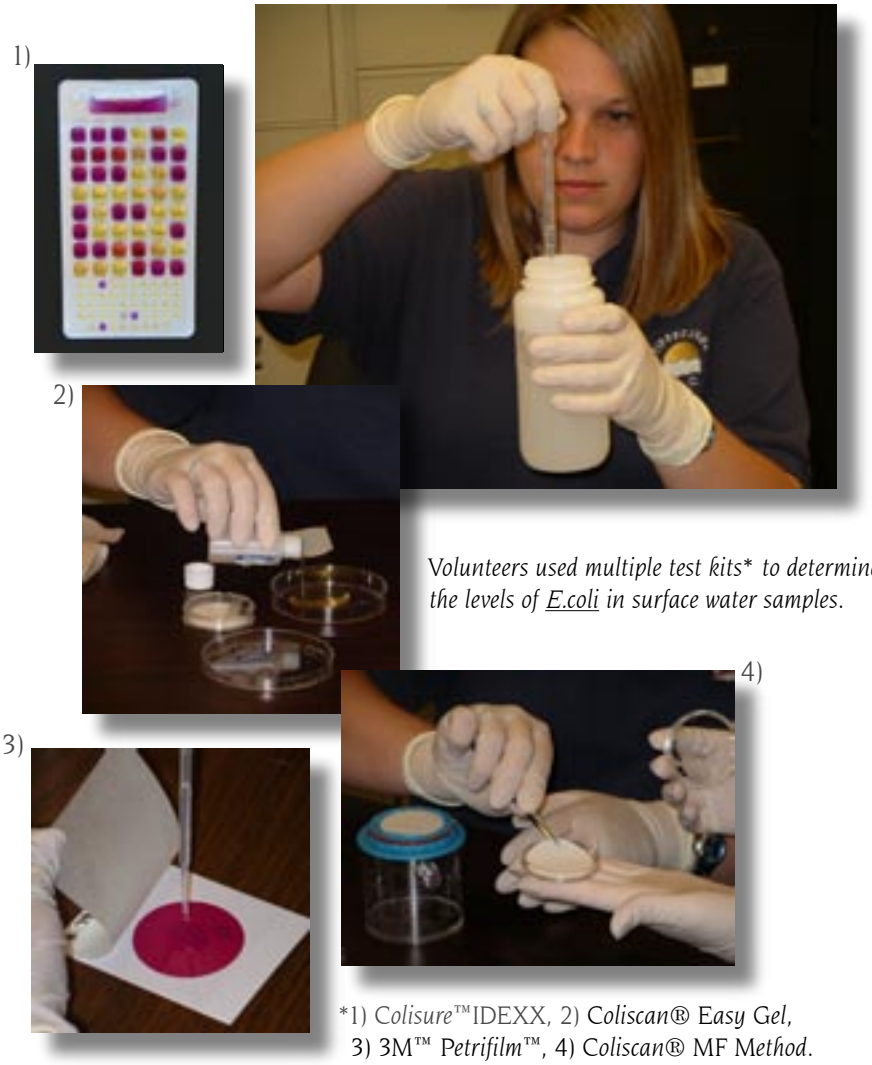
Test Kits

Coliscan® Easy Gel is the current method of choice for both the IOWATER Program and Hoosier Riverwatch. This test can be done by incubating the Petri dishes containing the sample water and media (as done by IOWATER) or leaving them at room temperature (as done by Hoosier Riverwatch). By adjusting the amount of surface water added to the media, this test can give very accurate results for total coliform bacteria and *E.coli*. The Coliscan® MF (Membrane Filtration) method is based on the same technology as Easy Gel, but rather than having surface water added directly to the media, larger volumes of water can be filtered through a membrane to gain more precision at lower levels of bacteria. In contrast, 3M™ Petrifilm only requires one milliliter of surface water to be placed on the Petrifilm™ media. Due to the inability to easily adjust the amount of water added during this test, precision may be lost at both extremely high and low levels of bacteria. The final method, IDEXX Colisure™, is a variation of a method approved by the United States Environmental Protection Agency for surface water. It employs statistical calculations to determine the "Most Probable Number" of bacteria present in water. This method allows any amount of water up to one hundred milliliters to be analyzed, therefore allowing greater flexibility at varying bacteria levels.

Method Specifics

Coliscan® Easy Gel (not incubated). This test kit, distributed by Micrology Laboratories, is being assessed in both Indiana and Iowa. To use the kit for analysis, a small amount (0.5-5 milliliters)

of a surface water sample is poured into a bottle of Coliscan® Easy Gel and mixed by swirling the bottle. The mixture is then poured into a labeled, pretreated Petri dish, covered, and gently swirled to uniformly distribute the sample. After sitting undisturbed for approximately 45 minutes, the Petri dish is inverted. The dish is then left at room temperature for 48 hours, at which time, purple/blue colonies (the *E.coli* colonies) on the dishes are counted. *E.coli* numbers are represented in colony forming units (CFU) / 100 milliliters of water.



Volunteers used multiple test kits to determine the levels of E.coli in surface water samples.*

*1) *Colisure*™IDEXX, 2) *Coliscan® Easy Gel*, 3) 3M™ *Petrifilm*™, 4) *Coliscan®* MF *Method*.

Coliscan® Easy Gel (incubated). This test kit, distributed by Micrology Laboratories, is being assessed in both Indiana and Iowa. To use the kit for analysis, a small amount (0.5-5 milliliters) of a surface water sample is poured into a bottle of Coliscan® Easy Gel and mixed by swirling the bottle. Then, the mixture is poured into a labeled, pretreated Petri dish, covered, and gently swirled to uniformly distribute the sample. After sitting undisturbed for approximately 45 minutes, the Petri dish is inverted and incubated at 35 degrees Celsius for 48 hours. Then, as with the non-incubated Easy Gel method, purple/blue colonies (the *E.coli* colonies) on the dishes are counted. *E.coli* numbers are represented in colony forming units (CFU) / 100 milliliters of water.

3M™ Petrifilm™. This test kit, distributed by 3M™, is being assessed in both Indiana and Iowa. To use the kit for analysis, the top film is lifted and 1 milliliter of a surface water sample is added to a Petrifilm™. The top of the film is then rolled down and all air bubbles are removed. Petrifilm™ is then incubated at 35 degrees Celsius for 48 hours. Blue colonies with gas are counted after both 24 and 48 hours to see how consistent the numbers are between incubation periods. *E.coli* numbers are represented in colony forming units (CFU) / 100 milliliters of water.

Coliscan® MF. This test kit is being assessed only by volunteers in Indiana. As with Easy Gel, this kit is distributed by Micrology Laboratories. To analyze for *E.coli* with this kit, a diluted surface water sample is poured through a filter. An agar-based medium is added to a Petri dish while the filter is

Training sessions will continue to focus on consistent training methods to increase knowledge and proficiency in volunteer monitoring of E.coli bacteria.

placed directly on the agar. The Petri dish containing the media and filter is then covered and incubated at 35 degrees Celsius for 48 hours before being read. Blue/purple colonies are counted as *E.coli*. In this method, *E.coli* numbers are represented in colony forming units (CFU) / 100 milliliters of water.

Colisure™ Method with the IDEXX Quanti-Tray®/2000. This test kit is being assessed only by Iowa volunteers and is distributed by IDEXX Laboratories. To analyze for *E.coli* with this kit, a packet of reagent is added to 100 milliliters of sample water or a diluted sample and mixed by shaking. The mixed sample is then poured into a Quanti-Tray® and sealed. The Quanti-Tray® is incubated at 35 degrees Celsius and read at both 24 and 48 hours. An ultraviolet light is used to determine the number of fluorescing, magenta wells in each tray. A combination of these two characteristics indicates the presence of *E.coli* within the well. A conversion table is used to determine the most probable number (MPN) of *E.coli* per 100 milliliters of water.

Acknowledgements

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Photos on pages 2-3 by Tippecanoe County (Indiana) Soil and Water Conservation District, except for page 3 top left photo by IOWATER. Page 4 photo by Indiana Department of Natural Resources Hoosier Riverwatch.

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Iowa Water Monitoring Program Web Site – wqm.igsb.uiowa.edu
Citizens Monitoring Bacteria – www.usawaterquality.org/volunteer/EColi/index.html

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