Bacteria Monitoring Protocol

April 2005

Muskoka

WATERSHED COUNCIL

BACTERIA SAMPLING PROTOCOL

Muskoka Watershed Council (MWC) publishes a watershed report card every three years with the first one produced in July 2004. The report card reports on Our Water, Our Air, Our Land and Our Community. An important component of the Water section is the swimability and drinkability of the water – bacteria level is a key indicator of this section.

The second report card is scheduled to be released in the summer of 2007. If the report card is to continue to report on swimability and drinkability in terms of bacteria, then consistent and comparable data are required. Associations can assist with this data requirement by collecting data in a standard manner and providing MWC with consistently reported results.

Where a lake association agrees to share their data with the Muskoka Watershed Council for inclusion in the Watershed Report Card, it is important that the following monitoring design and methodology be used.

The Muskoka Watershed Council has a limited interest in bacteria data and the individual lake association may wish to undertake a more comprehensive program. Also, the report card will only report on one indicator of bacteria, that being the average overall *E. coli* level as determined from sample sites submitted. Again, individual lake associations may wish to undertake more or different analytical tests. The Council in no way intends to limit a lake association that may wish to undertake a more robust and comprehensive program. The Council only wishes to obtain a limited amount of consistent data from across Muskoka for comparison purposes.

MONITORING DESIGN

<u>Research Question:</u>

1. What are the average bacteria levels at common recreational sites in waterbodies across Muskoka, where bacteria are defined as *E. coli*? Data will be considered from both the Beach Sampling program, undertaken by the Health Unit, and volunteer based lake ratepayer association programs.

<u>Methods</u>

Bacteria Analysis Methodology:

Once collected samples may be analyzed using either of the following two methods:

1. Lab analysis using a certified lab.
2. A ‘field test kit’, such as the coliplate.

Considerations:

1. Emphasis is on near-shore sites where the majority of recreational activity occurs, however, one (1) or two (2) deep water sites should be tested to establish background levels. These sites must be clearly identified.

2. Distribute sites randomly around the lake in association with a variety of residential and resort commercial uses. Natural sources of bacteria such as inflowing streams, wildlife or waterfowl congregation areas, and wetlands are not to be included in the study. One (1) or two (2) relatively undisturbed nearshore sites should also be tested to establish background levels. These sites must be clearly identified

3. Each year, sample in the same locations and be consistent with location names for easy identification. A map showing all sites should be provided to MWC to facilitate accurate reporting.

Sample Collection:

1. Samples are to be collected every two weeks starting on or before the July 1st weekend and ending after Labour Day. This allows periods of intensive use (long weekends) and average use to be captured. Sampling should occur on Mondays to facilitate quality control at labs. Sunday samplings are a problem on long weekends as the lab isn't open on the Monday.

2. One (1) or two (2) heavy rainfall events should be sampled. These should occur within 24 hours of a thunderstorm event. Heavy rain events should be flagged for easier identification and interpretation.

3. Samples are to be collected before 10:00 am on the scheduled sampling date to ensure a uniform collection timeframe.

4. All samples must be kept cool and in the dark. Lab samples must be delivered to the lab within 24 hours of sampling.

Quality Control:

Three quality control measures are required:

1. **Field Blank:** Use a distilled water sample to provide assurance that the sample bottles were sterilized properly, that the volunteer samplers were handling the bottles correctly, and that the 'field lab' volunteers processing the samples followed appropriate procedures. This field blank involves one volunteer filling a sample bottle in the field with distilled water during each sampling date. Label bottle as 'Field Blank'.

2. **Field Duplicate:** Use a field duplicate in the processing of one of the samples on each of the sampling dates in each 'field lab' station to ensure that the 'field lab' volunteers followed appropriate techniques. The field duplicate involves one volunteer filling a larger sample bottle with water from a pre-selected site; and the 'field lab' volunteer filling two ColiPlates (one for the site and one for the field duplicate) and the remaining volume of sample is refrigerated until it can be submitted to the accredited laboratory for standard analysis of Total Coliform and *E. coli* levels.

3. **Accredited Lab:** Use an accredited lab to determine the level of total Coliform and *E. coli*, in the sample used for the field duplicate for each volunteer 'field lab' station for each sampling date and thus confirm that the results determined by our volunteer 'field lab' stations using the 'Coliplates' are comparable to commercial lab results.

SAMPLING PROCEDURES

Equipment:

1. 2 m depth pole
2. Thermometer
3. Cooler
4. Ice Pack
5. 1 sterilized glass jar to collect water sample for each bacteria site and one spare jar as a backup.
6. Data collection sheets
7. Quality assurance material for sites –1 large sterilized bottle
8. A plastic bottle (obtained from and specific to the accredited laboratory being used for quality control checks).

Task One:

1. Verify that you have the appropriate boating safety equipment on board and that the weather is safe for sampling.

Task Two:

1. Record the air temperature as you leave the dock. Attach the thermometer to a rope on the boat.

Task Three:

1. Position the boat at the designated sample site. Water should be about ½ metre (1.5 feet) in depth.
2. Verify that you are at the right location by referring to the site photo and using the depth pole.
3. Be careful not to stir up sediment with the depth pole or the propeller. If conditions permit turn off the engine, particularly if it is shallow.
4. Record the water temperature.

Task Four:

Complete the observation portion of the sampling form noting the following:

1. Name of site,
2. Date,
3. Time of sampling,
4. Name of volunteer,
5. Weather conditions,
6. Recent precipitation,
7. Air and water temperature.

Record any other factors or conditions that make the sampling trip unusual or that may periodically influence sample results (cloudy water, unusual activity in the area).

Task Five:

Collect a sample for total coliform and *E. coli* analysis

1. Check that you have the bottle with the correct name for the site you are sampling. Remove the cap from the sterile collection bottle without touching the inside of the cap or the inside of the bottle.
2. Grip the bottle at the base and plunge it in a downward motion into the water to a depth of 22 to 30 cm (9 to 15 inches). The bottle goes into the water top down.

3. Use a forward sweeping motion (so water is not washed over the hand into the bottle) to collect the sample and bring the bottle to the surface.
4. Empty it slightly to leave some air at the top.
5. Re-cap the bottle, then check the label and store it in the cooler, with the ice pack, at a temperature of 4 to 8^0 C (39 to 45^0F)

QUALITY ASSURANCE PROCEDURES

Quality assurance is necessary to validate that the sampling and processing protocols have been followed appropriately. It is very important that these procedures are followed in order to ensure high quality results.

For each sampling date a volunteer is assigned the task of collecting both a field duplicate and a blank (distilled water) sample, which are used to confirm the scientific validity of the data.

Field Sample:

Using the large jar, take the bacteria sample in the same method outlined above. As soon as the sample is obtained, recap the jar and **shake it two or three time**, (this is necessary to ensure a uniform distribution of the discreet bacteria in the water sample). Immediately transfer some of the sample to the following containers:

1. The glass jar labeled for that site
2. The glass jar labeled as the field duplicate for that date
3. The plastic bottle for the sample to be analyzed by the accredited lab

Cap all three containers and place in the cooler with an ice pack, at temperature of 4 to 8^0 C (39 to 45^0F). Discard any remaining water in the large collection jar.

Distilled Water

After the site sample and field duplicate have been collected, open the distilled water jug and fill the glass bottle marked Distilled Water. Cap the glass bottle and place it in the cooler with the ice pack.

Lab Sample

The plastic bottle identified to be used for the lab sample must be refrigerated and delivered to the lab (on ice) for analysis within 24 hours of collection.

NOTE

All results for a sampling date will be discarded if the field blanks do not equal zero.

FIELD ANALYSIS (Coliplate - Processing the Samples)

Task 1: Preparation

1. Keep each sample refrigerated until you are about to fill the coliplate
2. Plug in incubator and bring it up to 35°C (95°F). Start this process at least one hour before samples are loaded. Place the thermometer on the bottom of the incubator in a position that can be easily seen through the viewing window.

Task 2: Fill Coliplate

1. Remove the samples from the fridge.
2. Match each sample with a coliplate by noting the site number on the label on the coliplate.
3. Fill all cells in the plate by pouring the sample water on the coliplate. If necessary, use a pipette to completely fill each cell; the pipette must be discarded after it is used in any cell as it cannot be sterilized for use on another cell or sample.
4. Closely examine the plate as it fills (preferably in front of a window) and check to ensure that all cells are full, and that there are no trapped air bubbles (readily apparent by the concave line in the cell). To get rid of trapped air bubbles, pour additional water over specific cell, and tap the coliplate briskly, taking care to ensure that your hands do NOT touch the interior of the plate (or its lid.)
5. Make sure that there is no surplus of water around the edges of the plate because it could contaminate cells; dry with a clean piece of paper towel or kleenex (do not repeat use of paper towel). The coliplate can be tilted on a 45^0 angle to facilitate draining of excess water.
6. Leave the filled and labeled coliplate on the counter until all samples are poured.

Task 3: Incubation

1. When all coliplates are filled, load them into the incubator; making sure that you can observe the temperature. Do not overload by stacking the plates two deep; this will affect airflow.
2. Replace the lid and allow the incubator's temperature to re-stabilize at 35 degrees Celsius. It may take an hour or so for this to occur.
3. Note the time when the incubator temperature is established.
4. Cover the windows with a paper to reduce the amount of natural light that may enter incubator and encourage the growth of algae in the cells.

Task 4: Incubation Time

1. Let the coliplates incubate for 26 hours from the stabilization time.
2. Once in a while check that the incubator is remaining at the appropriate temperature.
3. Minor adjustments may be necessary.
4. After 26 hours of incubation at 35°C (95°F), remove the coliplates from the incubator.

Task 5: Results

1. Total coliform count:
   a. For each coliplate, count the number of coloured cells that have turned blue or blue/green.
   b. Record this number on the site data sheet. (Placing the coliplates on a white piece of paper assists in assessing the number of coloured cells.)

2. *E. coli* counts:
    a. For each coliplate shine the UV light on it and count and record the number of BLUE cells (identified in step 1) that fluoresce. This is best done in a darkened area with the coliplate on a black background (i.e. sheet of black paper).
    b. Record this number.
    c. Repeat this process for all coliplates.

Task 6: Analysis

1. Using the Most Probable Number (MPN) chart, convert the cell counts to the most probable number of colony forming units (CFU) and record this number.

Task 7: Clean up

1. Sterilize all bacterial bottles by boiling jars and caps in water for 10 minutes. Be sure that all caps are indented once the jars are cooled thus confirming that they are sealed properly. (Jars often make a snapping noise when the vacuum pulls the caps to the indented position.)
2. Set up the jars for volunteers to pick up for the next sampling date.

DATA ANALYSIS

1. Record data in appropriate cell of Excel spreadsheet. Geometric will be calculated automatically for each site.
2. Count the number of occurrences of readings in each category, as defined on the summary sheet. Record number in the appropriate cell. The total at the end of the row should equal the number of sites for which you have sample data. This will confirm that you have entered and counted all sites.
3. At the end of the season, submit your results to the Muskoka Watershed Council at watershed@muskokaheritage.org