Swim Drink Fish Recreational Water Quality Monitoring Hubs

Standard Operating Procedures

Instructions for the Sample Collection and Laboratory Analysis of Total Coliforms and *Escherichia coli (E. coli)*

JUNE 1, 2022

SWIM DRINK FISH

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Introduction Standard Operating Procedures

Is the water clean enough for recreation?

At Swim Drink Fish Canada, our procedures help us answer the most popular question we receive from the public: *Is the water swimmable?* In other words, is the water clean enough for recreation?

Each time we monitor a beach, river, or other recreational water site, we ask ourselves:

- Are we confident that the samples truly represent the water at that location? (Have samples been taken carefully, without contamination, and handled properly?)
- Are we confident that the sampling and analysis methods are consistent, so we can compare them over time?

Volunteers follow procedures

This is the process required for bacterial monitoring in recreational water, from planning and preparation to water sampling, transportation, processing, analysis, and data management. It applies to all monitoring programs at Swim Drink Fish.

The expected outcome of this standard operating procedure is a set of test results for water quality with respect to bacteria. Test results are expressed as the Most Probable Number or colony forming units of *E. coli* per 100 mL of water (MPN or CFU per 100 mL).

The scope of these standard operating procedures is limited to general practices of volunteers and staff in the collection, transportation, processing, and analysis of samples. It does not include codes of practice relating to exposure of workers to biological or chemical agents. They also do not cover site-specific protocols and procedures such as a monitoring hub site's own regulations.

Training ensures consistency

Individuals must successfully demonstrate their ability to follow sampling and/or laboratory procedures under the supervision of the Hub Coordinator, Program Manager, Project Leader or designer.

All volunteers and staff must undergo a training program, which includes adherence to water sampling and laboratory procedures and a basic occupational health and safety awareness training program, including the identification of potential or existing hazards from water sampling and/or laboratory procedures. It also includes training on how to use personal protective equipment, sampling equipment, and appropriate clothing.

Training assessments include a field test and/or laboratory test.

In certain cases, volunteers and staff may be required to become certified in Standard First Aid (Level 1) and/or complete a worker education and training program for WHMIS.

All staff and volunteers will review current safety data sheets (SDS). They must also prepare and follow site safety assessments and detailed emergency response plans.

Specific situations (e.g., boats, confined entry, ice, off-road access, wildlife areas, parking in a manner that encroaches on the road) require additional safety training and equipment. In addition to this Standard Operating Procedure (SOP), staff and volunteers will also follow site-specific safety, protocols, and practices.

FOLLOW THE PROCEDURES

Practices that are permitted are described within these procedures. If a practice is not stated or described, it is not permitted. For example, a bucket sampler may not be used to collect bacteria samples, so it is not included in the procedures.

Swim Drink Fish staff adhere to IDEXX Laboratories procedures.

Swim Drink Fish staff also adhere to Health Canada's Guidelines for Canadian Recreational Water Quality, Third Edition (2012).

Swim Drink Fish staff must know and follow any additional procedures described in provincial standards or guidelines.

In Canada

Guidelines for Canadian Recreational Water Quality – Third Edition (2012)

In Ontario

Provincial Water Quality Objectives, 2014, Ontario Ministry of Environment

Recreational Water Protocol, 2018, Ontario Ministry of Health and Long Term Care

Operational Approaches for Recreational Water Guideline, 2018, Ontario Ministry of Health and Long Term Care

In British Columbia

Recreational Water Quality Guidelines, 2019, Government of British Columbia

In Alberta

2019 Recreational Water Monitoring for Beach Operators

Alberta Safe Beach Protocol, Alberta Health, Public Health and Compliance, Last Updated July 2019

Chapter 1: Sample Collection

1.1. Program planning (foundational and seasonal)

Before sampling activities begin, staff must take two essential steps:

1. Develop a data management plan - See *Chapter 4: Results Sharing* and *Appendix I* for more instructions.
2. Conduct Environmental Health and Safety Surveys (See *Appendix C*) for each sampling location in their program.

Environmental Health and Safety Surveys (EHSS) are one of the primary instruments used in protecting public health and safety on beaches and other natural recreational waters. EHSS are completed at the foundation of a sampling program for each recreational water location. They must be completed and updated at the start of every sampling season thereafter.

Annual EHSS reports assess the potential presence of chemical, physical, and biological hazards present at a specific recreational water use area by evaluating historical environmental observation and water quality data over time. When put together with routine environmental observations and water sampling, EHSSs form a multi-barrier approach to recreational water management.

Guidelines for Canadian Recreational Water Quality (2012) recommend that EHSSs be conducted by the "authority with the most knowledge of the day to day operations of the beach." This could be the appropriate provincial or territorial authority, the local beach manager, the local public or environmental health department, or informed and engaged community members.

For more instructions depending on your location, review the following materials:

- Health Canada's Guidelines for Canadian Recreational Water Quality (2012) include the checklist for the EHSS (Appendix C) and instructions on conducting the surveys (section 2.0).
- Ontario's Operational Approaches for Recreational Water Guideline, 2018 requires the completion of an annual survey and data review ahead of the bathing season.
- Alberta Safe Beach Protocol provides a site assessment tool to assess known and potential biological, physical, and chemical hazards, including any adjacent activities that may affect the site and their associated risks to the health and safety of the public.
- All of British Columbia's health units—Interior Health, Fraser Health, Island Health, and Vancouver Coastal Health—require the completion of EHSS reports according to the federal Guidelines for Canadian Recreational Water Quality (2012).

1.2. Safety is an essential part of water sampling and analysis

Use good judgement when collecting water samples

Always remember to be safe and use good judgement. It is beneficial and safer to work in groups of at least two when collecting water samples. Check in with the project manager at a pre-set time each day.

When thunder roars, go indoors

A sampler who hears thunder or sees lightning must stop immediately and return to their vehicle, a house, or other structure for shelter.

According to Environment Canada, “once you are in a safe location either in a house or a car, stay inside for 30 minutes after the last rumble of thunder. Every time you hear thunder rumble you need to restart the clock until 30 minutes has passed and there has been no thunder heard.”

Report unsafe conditions

Report any absence of or defect in any equipment or protective device to the Joint Occupational Health and Safety Committee. Follow Swim Drink Fish’s written occupational health and safety policy and program.

Start with good hand hygiene habits

Wash your hands following the proper methods of hand washing described in The Benefits of Hand Washing.

Vaccination is recommended

Get vaccinated against hepatitis B, polio, tetanus, and typhoid before handling polluted samples. There is a potential for environmental samples to contain untreated sewage, which may contain human pathogens. Protect yourself by ensuring your vaccines are up to date.

Use personal protective equipment in the field and laboratory

Use antibacterial gel on gloved hands. Such gel must contain at least 60% ethyl alcohol. Disinfectant wipes can also be used.

In the field, always wear:

- Clothing and footwear appropriate for conditions: Waders, water socks or shoes are required when entering a site from a beach.
- Personal protective equipment, devices, and clothing appropriate for the site and situation.
- If entering water deeper than 0.5 m a life jacket or PFD must be worn.

In the laboratory, always wear:

- a clean change of clothes (not field clothing);
- a laboratory coat;
- closed footwear (not open toes);
- goggles or safety glasses to protect eyes from contamination and UV radiation;
- other Personal Protective Equipment, as indicated on the SDS for all controlled substances.

Use antibacterial soap and/or gel to wash hands before and after laboratory work.

Can you swim?

Staff and volunteers should have the ability to swim if they are entering the water. If you do not know how to swim, you should not enter the water to collect samples.

Avoid the water when there are unsafe conditions

There are certain conditions when hub coordinators and volunteers must not enter the water and/or should suspend sample collection. Remember that hip-waders will become anchors if they fill up with water. Suspend sampling when any of the following conditions are present:

- large waves [>1.2m]
- rip currents
- high flow volumes
- swift moving water

Use your judgement. If conditions appear unsafe, do not enter the water to collect a sample.

Other common hazards in the field

Other common hazards/safety considerations require specific safety equipment and clothing to keep safe:

- cold water - wear waders PA
- risk of skin infection from snails, mussels, large amounts of algae or waterfowl - wear waders
- Abrasion from excessive zebra and/or quagga mussels - wear waders or water shoes
- extreme heat - remain hydrated, take breaks as needed, wear light coloured/light weight clothing
- risk of sunburn - wear sunscreen/wide brim hat
- bug bites - use insect repellant and always check for ticks at the end of each sample excursion in tick-prone areas. In tick-prone areas, samplers are also encouraged to shower at the end of a sampling session to remove ticks.

See *Becoming Familiar with Equipment* for a list of safety equipment.

Use environmental controls in the laboratory

Foot traffic through the laboratory is forbidden when samples are being processed:

- When delivering samples to the laboratory, knock and wait for a response before entering. Enter only when the technician is not processing samples.
- Keep the back door closed except in an emergency.
- Keep the front door closed except to allow the entry and exit of laboratory personnel.

Keep the laboratory clean:

- Keep out all food, beverages, and gum.
- Keep out all reusable bottles or utensils.
- Wet-mop floors and treat with a disinfection solution as needed; do not sweep or dry-mop.
- Maintain benches in a clear, uncluttered condition.
- Keep the refrigerator and incubator clean and free of all food and beverages.

Keep the laboratory disinfected:

- Wipe down countertops with a 1% bleach solution or methyl (or isopropyl) alcohol before and after use. Alternatively, clean aluminum foil can be laid over the work area before each use.
- If a culture of infectious material is spilled, disinfect and clean the area immediately.
- Keep all contaminated materials out of the sink and out of ordinary waste bins. Items known or thought to be contaminated (such as incubated Quanti-Trays) must be disposed of according to on-site regulations. As a best practice, we recommend placing contaminated materials in Stericycle boxes for later pickup and incineration.

USE ROUTINE PRACTICES IN THE LABORATORY

In the laboratory, always:

- Treat all samples as if they are potentially pathogenic.
- Keep materials and hands away from face and mouth;
- Keep conversation to a minimum.

1.3. Record-keeping demonstrates due diligence

It is important to develop a data management plan to increase the findability, accessibility, interoperability, and reusability of all data from the field and laboratory.

See Part V: What's your Data Management Plan? How to maintain, secure, and archive digital versions of all data

There are several record-keeping requirements

Keep diligent records at each hub, including:

- Sample Labels
- Chain of Custody Forms
- Environmental Observation Forms
- Field and laboratory photographs
- Lab Notebook
- Lab Bench Sheet
- Temp Logs Binder
- Reference tables:
    - Dilution Multiplication Table
    - IDEXX Quanti-Tray MPN Table
    - Confidence Intervals Binder

Table 1. Proper labels

Category	Description	Example
Field ID, Lab ID, & Sample ID	The format depends on whether the sample is part of a specific project. Regardless, IDs should be easy to understand. Document full ID explanations in the Quality Assurance Project Plan. *Project-specific format:* **Project Code** (4 or 5-characters): an acronym describing project or location **Station Number** (2-digits): indicates the station, as listed from east to west or in the order of the sampling route. **Sample Trip** (2-digits): indicates the sample trip in sequence or the week of the sampling season. **Modifier** (3-characters): indicates duplicate samples (e.g., DUP), dilutions (DIL), and follow-up samples (RS1, RS2, etc.)	MLOI-6A-01 (general sample from My Lake Ontario Inhouse MLOI, from the 6th station of route A, in the first week of the sample season) MLOI-6A-01-DUP (duplicate sample from above) MLOI-6A-01-RS1 (for the first follow-up re-sample collected) MLOI-6A-01-RS2 (for the second follow-up re-sample collected) MLOI-6A-01-RS2-DUP (for a duplicate sample taken with the second follow-up re-sample)
Sampler ID or name	Unique identification number for each individual involved in water sampling	

Technician ID or name	Unique identification number for each laboratory technician; may be the same as the sampler ID	

Chain of custody forms track each person who handles a sample

Sample custody must be traceable at each step from sample collection to disposal. It is helpful to minimize the number of people handling samples.

A sample is under custody if:

- It is in the individual's physical possession;
- It is in the individual's view, after being in their physical possession;
- It was in the individual's physical possession and is secured to prevent tampering; or
- It is placed in a designated secure area or secured in an area restricted to authorized personnel.

Table 2. Chain of Custody Events

Custody Event	Record-keeping	Explanation
Collection	Fill out a Chain of Custody form (Appendix B) for each sample or collection of samples that are handled and stored together.	All sample collection activities must be traceable to the person/people collecting the sample.
Hand-off	To hand off samples, review and sign the Chain of Custody form. Note date and time when samples were handed over. To accept samples, review and sign the Chain of Custody form. Note the date and time when samples were received. Check the temperature of the temperature blank to ensure samples are <10°C.	There must not be a lapse in time between the hand-off and received times on the Chain of Custody form. Samples must be kept between 0°C and 10°C until they are processed to be considered valid.
Other	Make corrections to the Chain of Custody form by marking one line through the incorrect information. Initial the correction (if it is corrected immediately).	

	Initial and date the correction (if it is corrected after the fact).	

1.4. In-field Sample Collection

Become familiar with equipment

Sampling requires attention to detail. Make sure to prepare equipment for sampling, including:

Sampling gear:

- Sample vessels (Sterile 100 mL bottle or Whirl-Pak©)
- Insulated cooler and ice to store and transport samples between 1°C and 10°C (ideally 4°C). Use wet ice in ziplock bags, or ice packs if wet ice is unavailable.
- Sample storage container for inside the cooler
- Sample blanks (Table 3)
- Bridge sampler and rope (as necessary, refer to Table 4)
- Pole sampler (as necessary, refer to Table 4)
- Water Rangers Kit for marine or freshwater
- Thermometer (X2)
- Camera

Safety gear:

- Smart phone
- First aid kit
- Retro-reflective safety vest (for use within the highway right-of-way)
- PFDs (for use on or near water)
- Other site- and situation-specific personal protective clothing, devices, and equipment

Proper clothing:

- Footwear
- Gloves
- Waders, water socks, or water shoes

Safe handling and disposal gear:

- Disposable gloves
- Antibacterial soap or gel
- Paper towels
- Wash water
- Plastic bag/s for waste

Paperwork:

- Chain of Custody Form
- Environmental Observation Form
- Waterproof pen and permanent marker
- Clipboards
- Site maps
- SOP
- Coordinator Booklet
- Volunteer Booklet

TABLE 3. HOW TO PREPARE QUALITY CONTROL AND QUALITY ASSURANCE SAMPLES

Type of Blank/QC	Planning & Preparation	Steps	Record-keeping	Safe Handling and Disposal	Explanation
Temperature blank	Attach label to sampling vessel or use a permanent marker to write sample ID on vessel or Whirl-Pak	**Temperature blank**: Fill a sampling vessel with water from the first sample site. Keep temperature blank "as-is" with no dilutions. Keep the temperature blank off the ice until you start collecting samples.	Label: "Temperature Blank" or "Temp Blank"	Seal the sampling vessel tightly and do not remove it from the cooler or open it again until you are back at the lab after sampling.	Sampling blanks will be used to check sample temperature upon delivery to the laboratory to ensure samples are between 0°C and 10°C. Temp blank should not be placed on the ice a long time ahead of the samples, as this can give an inaccurate representation of the sample temperatures.
Field Blank	Attach label to sampling vessel or use a permanent marker to write sample ID on vessel or Whirl-Pak	**Field blank**: Fill a sampling vessel with deionized water before leaving the laboratory. Keep field blank "as-is" with no dilutions. Exclude any samples that were in a cooler with a contaminated field blank (bacteria count greater than "<1").	Label: "Field Blank" or "QA Blank"	Use sterile pipette tips and pipettor to dispense water. Seal sampling vessel tightly and do not open it again.	Field blanks are used to determine if contamination has occurred during field sampling, storage, transportation, and processing. The results of the field blank should indicate that no bacteria is present and verify that no cross-contamination has occurred.
Laboratory Blank	Run a weekly blank for dilutions or when	**Laboratory blank:** Fill a sampling vessel with	Label: "Laboratory Blank"	Use sterile pipette tips and pipettor to dispense water.	To verify laboratory sterility, all blanks should read below the detection limit ("<1") for total coliform bacteria and *E. coli*.

	field blanks are not in use.	deionized water from the laboratory bottle. Keep laboratory blank "as-is" with no dilutions.	Record results in the Lab Notebook.	Thoroughly clean and sanitize all laboratory and field equipment if a laboratory or field blank indicates potential contamination.	Laboratory blanks can help identify the source of contamination (e.g., in the laboratory equipment or in the cooler).
Sample Duplicate	For every 10 water samples, you must take 1 field duplicate (10% duplicate samples)	**Sample Duplicate:** Use the pre-prepared randomized schedule for sample duplicates. Label the sample vessel with the appropriate ID.	Include "-DUP" as the suffix of the chosen sample site ID.	Follow section *Sample Collection Procedure* for the proper water sampling technique.	Duplicate samples help ensure the quality of our sampling procedures from start to finish. We are aiming for a 95% confidence interval with duplicates. The results are qualified whenever duplicate results are not statistically different (within the 95% confidence interval). Predetermined duplicates (by a random number generator) should be determined prior to sampling.

TABLE 4. SAMPLE COLLECTION GUIDELINES FOR RECREATIONAL WATER AREAS

Recreational water quality monitoring should be representative of the greatest recreational water activity at the monitored location. Special consideration must be given to the most vulnerable members of the population. Historically, samples were collected at a 1.2-1.5 metre (m) depth, the equivalent of the average male adult chest height. However, the most recent recreational water quality guidelines from the US Environmental Protection Agency and Health Canada now recommend sampling between knee and waist depth (0.15-0.5 m). The US EPA and Health Canada recommend this depth, because it represents the water that children and at-risk individuals will encounter during recreational water activities (US EPA 2012; Guidelines for Canadian Recreational Water Quality, 2012).

Recreational water locations vary from sandy beaches, to dock and pier locations, to rivers and swimming holes. This table serves as a site-by-site guide for Swim Drink Fish staff and volunteers collecting recreational water quality samples.

Site	Starting Location	Sampling Location	Direction of the Sampling Vessel	Depth	Troubleshooting	Typical safety hazards
Dock or Pier	Start by walking out to the end of the pier.	From the end of the pier, or as specified, facing the current	Move the bottle/Whirl-Pak away from the pier/dock and away from hands and body.	In water between 1.0m and 1.5m deep, collect samples 0.15m-0.30m below the surface. This is about the length of your forearm.	A water scoop or sampling pole may be used.	unstable floating docks, limited lifesaving equipment/ladders, high piers without railings, thunder and lightning
Shore samples (swimming beach)	Start by wading out into the recreational water area.	Collect the sample within the recreational area, between knee and waist deep (0.5m-1.0 m). Face the current.	Move the bottle away from hands and body.	Collect the sample within the bathing area, between knee depth and waist depth (0.5m-1 m). Face the current. Sample collection depth varies, but aim for 0.15m-0.30 m, between wrist and elbow.	Move slowly in the water. If sediment is disturbed, stand and wait until it settles.	large waves, rip currents, currents, cold water, dermal exposure to irritants, thunder and lightning

Wadeable Stream or river (A site where the sampler can wade in some distance.)	Start downstream of the intended sample location. Then, move into place.	Mid-stream, facing the current	Move the bottle away from hands and body.	If stream is <0.60m deep: mid-depth If stream is >0.60 m deep: 0.15-0.30 m below the surface	Consider using wading boots, hip waders, or chest waders. A sampling pole may be used. Use bridge sampling if access is limited (e.g., overgrowth, permissions).	swift currents, debris, cold water exposure, quickly changing water levels at certain times of the year, or during wet weather events, thunder and lightning
Boat	Start by moving the boat to the sampling location.	From the upstream side of the boat, facing the current	Move the bottle away from the boat and away from hands and body.	Variable, depending on local standards. In Ontario, in water between 1.0m and 1.5 m deep, collect samples 0.15m-0.30m below the surface. This is approximately the length of your forearm	A sampling pole may be used. It may be necessary to stop the engine or idle forward to avoid contaminating the sample with exhaust.	large waves, boat traffic, cold water exposure, thunder and lightning
Bridge	Park on the shoulder near the bridge and turn on vehicle hazard lights. Wear a reflective safety vest. Start by walking to the centre of the bridge.	Mid-stream, facing the current	Swing the bottle downstream. Pull the bottle into the current and up out of the water.	Variable, depending on local standards. In Ontario, in water between 1.0m and 1.5 m deep, collect the sample 0.15m-0.30m below the surface. This is approximately the length of a forearm	Vehicles on a bridge are dangerous. Use hazard lights, signage, and cones to provide advanced warning to motorists. Wear a retro-reflective safety vest for visibility. A bridge sampler and rope may be used.	thunder and lightning

TABLE 5. SAMPLE COLLECTION USING SAMPLING AIDS

Type of Sampling Aid	Steps	Explanation
Sampling Pole	Pole samples require a procedure to avoid cross-contaminating between sites. If the site has flowing water, you can dip the pole/rope sampler into the target water multiple times (x3) to limit cross contamination.	Used when it is necessary to collect a sample that is difficult to reach (from a point that is up to 3m away).
Bridge Sampler and Rope	Rope samples require a procedure to avoid cross contamination between sites. If the site has flowing water, you can dip the pole/rope sampler into the target water multiple times (x3) to limit cross contamination. Tie the end of the rope to the bridge rail. Lower the sampler and rope, avoiding the bridge and keeping it up off the stream bed.	These steps ensure that the sampler is secure and minimize contamination from debris.

SAMPLE COLLECTION PROCEDURE

STEP 1 BEFORE YOU HEAD INTO THE FIELD

Planning and Preparation

1. Follow your checklist to ensure you have all necessary equipment and paperwork packed. (See *Become Familiar with Equipment, p. 14*).
2. Prepare a cooler with ice. Use ice bagged in ziplock bags or ice packs.
3. Prepare a field blank by pouring 100 mL of distilled/deionized water into the correctly labeled sampling vessel (See *Table 1*).
4. Pre label all sampling vessels with location ID, site ID, replicate number, and type of sample (duplicate, field blank, or temperature blank).
5. Communicate to other Swim Drink Fish staff regarding the proposed start and end times, the sampling locations, and contact information while in the field. Ensure that Swim Drink Fish field staff have charged and working smartphones for data collection and in case of emergency.

6. Plan ahead to ensure that samples are delivered to the lab within 3 hours of collection. For non-potable water compliance, there is an 8-hour allowance from the time of collection to incubation.

Step 2 Sample Collection in the Field

Sampling at a Beach or Marina

1. Upon arrival at the site, check the site map to locate the predetermined sites.
2. Start filling out the chain of custody form, i.e., survey start, survey start air temperature, start wind direction and speed, etc.
3. Start filling out the environmental observations forms, i.e., wildlife, recreational water users, potential sources of contamination, etc.
4. Prior to sampling, ensure that all personal protective equipment (PFD, waders, water shoes, gloves) are on.
5. At the beach carefully wade into the water to a depth of 0.5m-1m knee to waist deep. At the marina kneel or lay down on the dock at the sampling point.
    a. Try not to disturb the sediment. If the sediment is disturbed, wait until the sediment settles.
    b. Place the thermometer into the water.
6. Wearing gloves, prepare a temperature blank (see *Table 1*) by following section *Sampling with a Whirl-Pak or Bottle*
7. Start sampling the first sampling location. Follow section *Sampling with a Whirl-Pak or Bottle* to sample the water.
8. At the same time, record the sample ID and the sampling time.
9. Once finished sampling, record the water temperature.
10. Using the secchi disc, measure the water clarity and depth. Record the values on the sample collection data sheet (Appendix B).
11. Safely exit the water and continue to the next site.
12. Continue sampling (following steps from section *Sampling with a Whirl-Pak or Bottle)* until water samples for all site locations have been collected.
13. Once all sampling locations have been sampled, complete the survey end section of Chain of Custody.
14. Ensure that all environmental observations are filled out.
15. Safely dispose of gloves in the waste bag.
16. Sanitize hands.

Sample collection using a sampling pole

1. Upon arrival at the site, check the site map to locate the predetermined sites.
2. Start filling out the chain of custody form, i.e., survey start, survey start air temperature, start wind direction and speed, etc.
3. Start filling out the environmental observations forms, i.e., wildlife, recreational water users, potential sources of contamination, etc.
4. Prior to sampling, ensure that all personal protective equipment (PFD, waders, water shoes, gloves) are on. Expand pole to the desired length.
5. Rinse the sampling side of the pole with the targeted water (three times).

6. Start by preparing a temperature blank (see *Table 1*) by firmly attaching the temperature blank Whirl-Pak to the sampling end of the sampling pole (using the C-clip).
    a. Once attached, tug on the Whirl-Pak bag to ensure that it is secure.
7. Sample the water by submerging the Whirl-Pak 0.15-0.30 m below the surface. Move the vessel horizontally in the direction it is pointed to help fill the vessel.
8. Carefully bring back the Whirl-Pak and remove without touching the inside of the vessel.
9. Follow Section *Sampling with a Whir-Pak* steps 5-7 or *Sampling with a Bottle* steps 2-5.
10. Once the sample is safely in the sample storage container in the cooler, place the thermometer into the water.
11. Begin sampling at the first site at the same time. Make sure to record the sample ID and the sampling time in the field data sheets or beach manager pro.
12. Once the sample is safely in the storage container in the cooler, record the water temperature on the data sheet.
13. Record the clarity and depth of water with the secchi disc on the data.
14. Move to the remaining sampling locations following the sample procedures as above.
15. Once all sites have been sampled, complete the survey end section of Chain of Custody.
16. Ensure that all environmental observations are filled out.
17. Safely dispose of gloves in the waste bag.
18. Sanitize hands.

Sampling with a Whirl-Pak

1. Put on gloves and disinfect the gloves with disinfectant.
2. Open the sampling vessel by removing the seal at the top of the Whirl-Pak.
3. Pull the side tabs to open the Whirl-Pak. Avoid touching the inside with gloved hands.
    a. If the inside of the Whirl-Pak is touched, it should be disposed of and a new bag used.
4. Submerge the Whirl-Pak 0.15-0.30 m below the surface. Move the vessel horizontally in the direction it is pointed to aid in filling the bag.
5. Aim to fill the Whirl-Pak between the 100 mL and 118 mL line.
6. Hold the Whirl-Pak by the wire ends and whirl it at least 3 times. Ensure that there is a head space (air) in the Whirl-Pak. Twist the two wire tags downwards and firmly together.
7. Invert the Whirl-Pak to ensure that there are no leaks.
8. Place the Whirl-Pak sample upright into the sample storage container inside the cooler. Samples should not be put directly against the ice or ice packs.
9. Once finished sampling, record the water temperature.
10. Using the secchi disc, measure the water clarity and depth. Record the values on the sample collection data sheet (Appendix B).
11. Remove gloves and sanitize your hands as necessary with the provided hand sanitizer.

Sampling with a sterile bottle from a lab

1. Put on gloves and disinfect the gloves with disinfectant.
2. Open the sterile bottle, breaking the sterility seal around the lid. Avoid touching the inside of the bottle and lid. Do not place the lid on the ground; it must be kept in a gloved hand.
    a. If the inside of the bottle or lid is touched it should be disposed of and a new bottle used.
3. Submerge the bottle 0.15 m to 0.30 m below the surface. The bottle will start to fill. Aim to fill the bottle to slightly above the 100 mL line. If it is overfilled, shake out the water until it reaches the 100 mL line.
4. When the water level reaches 100 mL it is ready to be capped. Cap the bottle tightly.
5. Store the sample in the cooler with ice to keep it at the right temperature range.
6. Remove gloves and sanitize your hands as necessary with the provided hand sanitizer.

Chapter 2: Sample Processing

WATER SAMPLES ARE PREPARED AND PROCESSED IN THE LABORATORY

2.1. PREPARING WATER SAMPLES (SWIM DRINK FISH STAFF/LABORATORY PERSONNEL ONLY)

Planning and Preparation

Samples must be processed within 8 hours of the time of collection. Samples should be processed within 2 hours of delivery to the lab.

1. Turn on the incubator and verify it is set to 35°C about two (2) hours ahead. Depending on what type and make, you may need to turn on the incubator before you head into the field. Keep in mind that incubators take over an hour to fully stabilize in temperature.
2. Turn on the IDEXX Sealer and preheat until the green indicator light is visible (approximately 10 minutes). Refurbished sealers can take up to 30 minutes to preheat.

Accepting Samples and Chain of Custody

1. Transfer samples, review and sign the Chain of Custody form. There must not be a lapse in time between the relinquished and received times on the Chain of Custody form.
2. Note date and time when samples were relinquished.
3. Inspect coolers. Verify the number of samples.
4. To accept samples, review and sign the Chain of Custody form.
5. Note the date and time when samples were received.
6. Note the temperature of the temperature blank. Qualify the sample if the temperature is between 0°C and 10°C. See *FAQ* for what to do if temperatures fall outside the 0°C-10°C range.
7. Place Chain of Custody forms in binder.
8. If holding time is >1 hour: Store samples in the laboratory refrigerator (2°C-8°C) in order to ensure samples are kept at <10°C. Keep a thermometer in a glass of water in the refrigerator to check temperature daily.
9. If the holding time is >6 hours: Qualify all results and exclude from public reporting.

2.2. PROCESSING WATER SAMPLES USING QUANTI-TRAY* ENUMERATION PROCEDURE

Swim Drink Fish follows IDEXX Standard Operational Procedures for processing and analysis of samples.

Preparation

1. Wipe down countertops/work area with a 1% bleach solution or methyl (or isopropyl) alcohol. Alternatively, clean aluminum foil can be laid over the work area before each use.
2. Wear gloves:
    - Once worn, lather the glove surface with sanitizer.
    - Allow to air dry.
    - Dispose of any gloves that come into contact with sample water and replace with a new pair.
3. Label back Quanti-Trays using a permanent marker, with the following information:
    - Sample collection date and time
    - Location name
    - Sample ID (in the “Sample #” field)
    - Dilution ratio, if applicable.
4. Place samples into blue racks on the lab bench, or into a similar tray or vessel, in labelling order (e.g., 1,2,3, A,B,C)
5. Ensure there is enough Colilert for all samples, including duplicates and dilutions.
6. Refer to Dilution Guide in Table 6 (p. 23) for sample quantity.

Add Colilert

7. Add the contents from a pack of Colilert to a 100 mL water sample in a sterile vessel (bottle or Whirl-Pak). When handling IDEXX disposable bottle, place cap on counter such that the inside of the cap is facing upwards.
8. Tap the Colilert capsule 3 times to make sure all the powder is released.
9. Cap vessel or close Whirl-Pak. Shake until Colilert powder is completely dissolved. Allow any foam to subside.

Diluting water samples for use with the Quanti-Tray* or Quanti-Tray*/2000 system

The maximum counting ranges for Quanti-Tray and Quanti-Tray/2000 greatly reduce the need for manual dilutions. However, if you suspect that a sample is heavily contaminated, follow these guidelines to dilute the sample before testing:

Dilute the sample if the expected MPN value, based on the MPN table included in the Quanti-Tray insert, is: >2,419 per 100 mL sample for the 97-well Quanti-Tray/2000

Dilute the sample before adding the Colilert*, Colilert*-18, Colisure*, Pseudalert*, Enterolert*, Enterolert*-DW, or HPC for Quanti-Tray* reagent to the sample.

Always use sterile deionized (DI) or distilled water as the diluent, as described in the package insert.

TABLE 6. RECOMMENDED DILUTION RATIOS

Dilution Ratio	Sample	Diluent	dilution factor (to use when calculating result)
1:1	50 mL	50 mL	x 2
1:4	25 mL	75 mL	x 4
1:5	20 mL	80 mL	x 5
1:10	10 mL	90 mL	x 10
1:20	5 mL	95 mL	x 20

Dilution instructions

1. Determine the ratio of the dilution. See table above. Determine the amounts of sample water and diluent you will need for your dilution. For example, for a 1:10 dilution, you will need 10 mL from the sample and 90 mL of DI water.
2. Label the new vessels for the diluted samples with the matching sample identifier and -DIL at the end and the Dilution Ratio. Prepare the first dilution vessel by removing the cap or opening the Whirl-Pak. Note: make sure that the Quanti-Tray labels on the back include the dilution ratio and -DIL on sample identifiers.
3. Add the related mL of sterile DI water to the vessel (e.g., for a 1:10 dilution, add 90 mL of DI water). Note: Diluent should be added to the vessel first, followed by the sample.
4. Mix the original sample thoroughly and then open the vessel and withdraw the appropriate amount of water from the sample using a pipette (instrument) with a sterile pipette tip, or equivalent.
5. Transfer the sample to the new sterile vessel with the diluent, such as a bottle or Whirl-Pak (e.g., for a 1:10 dilution you will withdraw 10 mL using a sterile pipette). Note: If using a Whirl-Pak, it helps to stand the Whirl-Pak in a container to keep it upright during dilution.
6. Add a packet of Colilert, to the diluted sample and mix well to dissolve. You can follow the same sample instructions for processing samples as for undiluted samples, as described in *Lab Step 2: Processing Water Samples - Adding Colilert.*
7. Pour sample into a Quanti-Tray or Quanti-Tray/2000, seal, and then incubate following the instructions in the package insert.

Pour the sample into the Quanti-Tray

10. Match the sample with the Quanti-tray with the same label.

11. Use one hand to hold a Quanti-Tray 2000 upright with the well side facing the palm and the foil side facing away from the palm.
12. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends towards the palm making a C shape.
13. Gently pull the foil tab up and out to separate the foil from the tray. Avoid touching the inside of the foil or tray. Dispose of the tray and start over with a new tray if the inside of the tray is touched or the tab is ripped.
14. Pour the solution into the Quanti-Tray, avoiding contact with the foil tab and the inside of the Quanti-Tray.
15. Relieve pressure on the tray to close the top.
16. Tap or flick the bottom edge of the tray two to three times to release any air bubbles

Seal Quanti-Tray with the IDEXX Sealer

17. Carefully place the Quanti-Tray into the rubber insert (white labelled side up). Make sure that the tray is flush against the rubber insert and each well is aligned into the holes of the rubber insert.
18. Feed this into the sealer, lifting the back end of the rubber insert if necessary. The white labelled side of the tray back should face up, and the wells should face down. The tray opening should face away from the sealer, going through the sealer last.
19. The sealer will start to draw in the Quanti-Tray. Wait approximately 5 seconds for the tray to move through and seal and eject from the rear of the sealer. You will need to gently pull the sealed tray from the sealer rear.
20. Check to ensure that all the wells are full. If more than 2 wells are empty, the sample is not valid. One or two empty wells do not affect the test interpretation as long as the entire sample is in the tray. The effect on the Most Probable Number (MPN) is statistically insignificant. A partially filled well is interpreted the same way as a full well. Go to the FAQ section for questions regarding partially full or empty wells
21. Repeat steps 14-17 until all Quanti-Trays are sealed.
22. Use the reverse button on the IDEXX Sealer only when the rubber insert is not fully drawn into the slot.
23. If a tray is overfull and spills out, rinse and dry the rubber insert before proceeding to seal the next sample.
24. Prepare a new sample if the tray is stuck or ripped.
25. Turn off sealer when finished.

Incubate

26. Ensure that the incubation of all samples begins within 30 minutes of adding Colilert to the sample and within 2 hours of delivery to the lab. Place the sealed trays in a 35±0.5°C incubator. The incubator must be capable of maintaining temperatures within ±0.5°C of the required incubation temperature of the method.
27. Plan for the following incubation times:
    - Colilert-18: 18 to 22 hours
    - Colilert: 24 to 28 hours
28. Stack the sealed Quanti-Trays in the incubator, ensuring that there is adequate space for air circulation. Keep stacks to a maximum of 8-10 trays high.
    - Binder capacity: 40 trays
    - Quincy capacity: 20 trays

29. If available, turn on a data logger to record incubator temperature for the duration of the incubation period or verify twice daily with a thermometer.
30. Fill our Lab Bench Sheet (Appendix D) with the following information:
    - Incubation start date and time
    - Dilution ratio, if applicable
    - Technician name or sample ID
31. After the allotted time (see *Incubate step 25*), fill out the Lab Bench Sheet (*Appendix D*) with the following information:
    - Incubation end date and end time
    - Note any time a tray is returned for extended incubation.
32. Remove samples from the incubator, leave the incubator on.

2.3. Clean and store equipment

After all samples are processed, wash coolers and sample storage containers with a mild detergent, rinse with tap water, and wipe down or air dry before storage. This is important, as the die-off and growth potential increases the more the time elapses between sample collection and processing. Follow the cleaning instructions in *Appendix E*.

Chapter 3: Sample Analysis

3.1. Reading Results (Laboratory Personnel Only)

Complete all tray counts within 30 minutes of incubation end time.

1. Remove the trays from the incubator and note the time of removal.
2. Before analyzing the Quanti-Tray, relabel the well side with a permanent marker at the top with the Sample ID and date. Begin analyzing.
3. **Total coliform bacteria count:** Using the comparator as a guide, count and record the number of positive large and small wells in the Quanti-Tray. Wells are positive for total coliform when they are equal to or more yellow than the wells in the comparator, even if the wells are not completely full.
    - Mark the positive yellow wells with a diagonal line in permanent marker as you count.
    - Count the large positive wells and record the number at the top right of the Quanti-Tray and in the Lab Bench Sheet.
    - Count the small positive wells and record the number at the bottom right of the Quanti-Tray and in the Lab Bench Sheet.
4. **Total *E. coli* count:** Place the sample tray into the black-light box. Turn on the 6 watt 365 nm UV lamp.
5. Compare each well with the comparator. Wells are positive for total *E. coli* when glowing under UV light and when they have been positively identified for total coliform, even if the wells are not completely full. Count and record the number of positive large and small wells.
    - As you count, mark the positive wells with a diagonal line (creating an X if the well is positive for both total coliform and total *E. coli*) in permanent ink.
    - Count the large positive wells marked with an X and record the number at the top left of the Quanti-Tray and in the Lab Bench Sheet.
    - Count the small positive wells marked with an X and record the number at the bottom left of the Quanti-Tray and in the Lab Bench Sheet.
6. Record results on the Lab Bench Sheet, including the following:
    - Sample ID
    - Number of positive large and small wells (for both total coliform and total *E. coli*)
7. Use the appropriate MPN chart (for Quanti-Tray or Quanti-Tray/2000) with the # of positive large and small wells to obtain the MPN for total coliform or total *E. coli* per 100 mL.
    - To correct the result for dilution, multiply the MPN value by the dilution factor (20) (table 6). Correct for dilutions of each sample, calculate the geomean using corrected single sample MPN results.
8. Record the MPN results (for both total coliform and total *E. coli*) on the Lab Bench Sheet.
    - Repeat counts for the same technician should be within 5%.
    - Counts between technicians should be within 10%.
9. Turn off the incubator when there are no more samples to process that day.

3.2. Checking Results (Laboratory Personnel Only)

1. If possible, print off data logger reports and verify that a proper incubation temperature of 35° C +/- 0.5° C was maintained.
2. Compare and record the MPN values of the duplicate samples on the Lab Bench Sheet
    - Check if the sample results are statistically different using the 95% confidence interval (See *Appendix H: IDEXX 95% Confidence Limits*).
    - Whenever duplicate sample results are statistically different, qualify all samples collected by that sampler on that sampling effort.
    - Exclude qualified results in public reporting programs such as Swim Guide.

3.3. Photographs and Wrap-Up (Laboratory Personnel Only)

1. Write the ID and sample date of the first sample tray on a small dry-erase board. Take photographs of the Field Blanks.
2. Photograph the sample tray with the dry-erase board
    - If the MPN for *E. coli* is > 100, photograph the tray under the UV lamp (UV light on) with the lab lights off.
3. Photograph the rest of the samples.
4. File the images in the appropriate digital folder in the Datakeeper database. Ensure that the quality of the photo is enough for a recount.
    - Recounts from the digital image should result in the same number as the first count.
5. Scan the Lab Bench Sheet and store it in the appropriate digital folder in the Datakeeper database.
6. Clean the incubator when it has cooled (*Appendix E*).
7. Following the cleaning SOP and schedule (*Appendix E*), clean up the lab space.

Note:

- Disposal requirements can vary. Check with your local, state/provincial, and/or federal authorities for proper disposal of bacteriological biohazard materials at your facility.
- It is the laboratory's responsibility to comply with all federal, state/provincial, and local regulations governing waste management, particularly the biohazard and hazardous identification rules and land disposal regulations. Compliance with all sewage discharge permits and regulations is also required.
- Samples, reference materials, and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal.

Chapter 4: Results Sharing

4.1. What's your data Management Plan? How to maintain, secure, and archive digital versions of all data

You've invested so much time and effort into establishing an incredible monitoring program. Data management ensures that your important work is protected, preserved and, most importantly, that it is accessible, useful, usable, and used.

Typically, field data is collected on paper data entry sheets, as is much of the laboratory data related to sample processing. A critical step in water quality monitoring is ensuring that all data, from collection to processing and analysis, is managed and stored properly online. If data is not managed properly, you run a high risk of it becoming unusable by creating data chaos, having poor data quality and incoherence, and of potentially losing your data.

From Data Collection to Digital Management

All field and laboratory data should be entered into an online database as part of regular monitoring activities. Online data entry should happen at the end of each sampling procedure (sample collection, sample processing, and sample analysis).

Structuring your data

Create an instruction document for the data management plan for the collection, storage, and sharing of your database prior to collecting samples and environmental data. This instructional document will be the foundation of how your data is organized and formatted, and it will determine which data fields should be included in your spreadsheets. Consistency in how data is structured and in how metadata is written is imperative. New to data? Watch this video.

See *Appendix I* for more instructions and a sample of the template tabular data spreadsheet after watching the Data 101 video.

Storing your data

Never, ever store your data in hardcopy (paper) only. You run a high risk of losing your data if it is stored on paper in a physical place. You should also avoid using applications on personal computers without a cloud-based or online backup and an external backup.

Your EHSS should also be online. Online databases are a good option for ensuring your data doesn't get lost. Google Drive's Google Sheets is a great option. For example, if you are storing the data on a Microsoft Excel spreadsheet or another application on a work or personal computer, we highly recommend you also upload to an online or cloud-based database and save it externally to a server like Google Drive.

MANAGEMENT/STORAGE

Data management is critical to the integrity of the water quality monitoring program. Review and verify all logs, records, and final reports with the Project Leader or their designee before releasing it to the public or private individuals.

Input digital versions of all data into the Datakeeper database and store a backup copy securely offsite. All databases should be frequently backed up. We recommend backing up all databases online, e.g., on the Cloud, Drop-Box, or Google Drive.

There should be both hard copies and digital copies of the field and the lab bench sheets. Hard copies should be scanned and placed into the appropriate folder in the Datakeeper database.

4.2. SHARING YOUR TEST RESULTS WITH THE PUBLIC

As test results become available, it is important that they are shared as soon as possible with the public. Swim Drink Fish shares results on its platform as interpreted data, meaning we share beach status as having met or failed to meet local water quality standards.

Swim Drink Fish shares test results on numerous channels including:

- Swim Guide website
- Swim Guide iOS and Android apps
- Great Lakes Guide
- Swim Drink Fish social media channels

Our open data is shared via our open data portal at recreationalwater.ca

Partner organizations are encouraged to share test results on their own channels and in multiple formats.

More about data sharing

Recreational water quality data is meant to be shared with the public so that they can protect their health from poor water quality and find great places to swim. As your test results become available, please ensure that they are shared on Swim Guide via your Beach Manager account. (Don't have a Beach Manager account? Reach out to the Swim Guide program manager at gabrielle@swimdrinkfish.ca). You should also ensure that the public has access to a raw (complete), machine readable dataset through an accessible platform, like an open data portal or your public facing website.

When sharing, we encourage that all data, including your EHSS and field data, is online and findable. Data published on open data portals should be easily accessible and should be made in a machine readable format, i.e., CSV, XML, or JSON.

References

Guidelines for Canadian Recreational Water Quality, third edition. Health Canada, April 2012. Available from: https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadianrecreational-water-quality-third-edition.html

National Beach Guidance and Required Performance Criteria for Grants, 2014 Edition. United States Environmental Protection Agency, July 2014. Available from: https://www.epa.gov/sites/production/files/2014-07/documents/beach-guidance-final-2014.pdf

Operational Approaches for Recreational Water Guideline, 2018, Population and Public Health Division, Ministry of Health and Long Term Care, Government of Ontario, 1 January 2018, Available from: http://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/protocols_guidelines/Operational_Approaches_to_Rec_Water_Guideline_2018_en.pdf

Recreational Water Protocol, 2018, Population and Public Health Division, Ministry of Health and Long Term Care, Government of Ontario, 1 January 2018, Available from: http://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/protocols_guidelines/Recreational_Water%20Protocol_2018_en.pdf

“Lightning in Canada: frequently asked questions.” *Government of Canada.* 2018, www.canada.ca/en/environment-climate-change/services/lightning/frequently-asked-questions.html. Accessed Feb 2019.

“OH&S legislation, Occupational Health and Safety Act, R.S.O. 1990, c. O.1.” *Ontario*. 2018. https://www.ontario.ca/laws/statute/90o01. Accessed Feb 2019.

“Occupational Health and Safety Awareness and Training.” *Ontario*. 2017. https://www.ontario.ca/laws/regulation/130297. Accessed Feb 2019.

“Workplace Hazardous Materials Information System (WHMIS).” *Ontario.* 2019. https://www.ontario.ca/laws/regulation/900860. Accessed Feb 2019.

"Control of exposure to biological or chemical agents." *Ontario.* 2019. https://www.ontario.ca/laws/regulation/900833. Accessed Feb 2019.

Protocols Manual for Water Quality Sampling in Canada. Canadian Council of Ministers of the Environment, 2011.

Standard Operating Procedures for Bacteriological Monitoring of Coosa Riverkeeper, Inc. Coosa Riverkeeper, 2016

Appendix A: List of Acronyms and Abbreviations

±	Plus-minus
°C	Celcius
<	Less than
>	Greater than
CFU	Colony forming units
cm/sec	Centimetres per second
CSV	Comma-separated values file
DD.DD, DD.DD	Decimal Degrees
DI	Deionized
DIL	Dilution
DUP	Duplicate
E. coli	*Escherichia coli*
EHSS	Environmental Health and Safety Sheet
FAQ	Frequently Asked Questions
GPS	Global Positioning System
HH:MM:SS	Hours:minutes:seconds
ID	Identification
JSON	JavaScript Object Notation
M	Meter
MF	Membrane filtration
mL	Mililitre
mm	millimeter
MPN	Most Probably Number
MTF	Multiple-tube fermentation
MUG	4-methyl-umbelliferyl- Bd-glucoronide
nm	Nanometre
OH&S	Occupational health and safety
PFD	Personal Floatation Device
QA	Quality assurance
QC	Quality control
Swim Drink Fish	Swim Drink Fish
SDS	Safety data sheet
SOP	Standard Operating Procedures
TEMP	Temperature
UV	Ultraviolet
WHMIS	Workplace Hazardous Materials Information System
XML	Extensible Markup Language
YYYY-MM-DD	Year-Month-Day

Appendix B: Swim Drink Fish Field Survey and Chain of Custody Form

Water Quality Sampling Chain of Custody Record

- Samples must be kept cold **(below 10°C) and dark** until delivery to Swim Drink Fish lab.
- Samples must be delivered to the Swim Drink Fish office **within 3 hours** of sample collection.
- If you have any questions or require assistance, please call (416) 861-1237.
- Please see support materials for instructions.

Send to:
Lab address here

Sample Date: _____

Survey start time: _____

Samples collected by: _____

Samples delivered by: _____

Survey end time: _____

Time of delivery to lab: _____

Samples kept on ice at time of delivery? (Y/N)

Temperature Blank temperature at the lab (C°): ___

Weather station information (station name, location (GPS coordinates), and distance from the sampling location from which you are obtaining wind speed/direction data if it is not being collected onsite):

Weather data			**Precipitation data**			
Metro Parameters	Survey **start** time	Survey **end** time	Rainfall	Did it rain? (yes/no)	Rain intensity: (misting/light/steady/ heavy/none/don't know)	Amount (mm)
Air Temperature °C			< 24 hours			
Wind Speed km/hr			< 48 hours			
Wind Gust km/hr			< 72 hours			
Wind direction (N,E,S,W) *e.g., NNE*			> 72 hours since last rainfall event			

Barometric Pressure (kPa)			**Notes:**	

Instructions:

- **Put on the gloves provided.** Initial here confirming that the sample was taken while wearing gloves: _____
- Measure the **water temperature** using the thermometer. Make sure to submerge in water for at least 30 seconds.
- Obtain and record the **time** of sample collection, **latitude and longitude** (GPS coordinates – can use Google Maps).
- Assess and record observations of the **water clarity** at this site (clear, cloudy, or turbid).

Add any **additional notes** (e.g., scum on the water; natural debris on water surface; water has fishy odour, etc.).

Sampler's name: _____ Date: _____

SAMPLE ID #	**TIME (00:00)**	**WATER TEMP (°C)**	**CLARITY and depth (secchi disc)**	**Visual Location** *(reference the map provided)*

WATER RANGERS DATA		
Environmental Observation	**Water type**	**Results**
Water temperature (thermometer)	Marine / Fresh	
Total Chlorine (test strip)	Fresh	
Alkalinity (test strip)	Marine / Fresh	
pH (test strip)	Marine / Fresh	
Total hardness (test strip)	Fresh	
Conductivity (conductivity meter)	Fresh	
Dissolved oxygen (dissolved oxygen kit)	Fresh	
Secchi depth (secchi disc)	Marine / Fresh	
Depth (secchi disc)	Marine / Fresh	
Salinity (refractometer)	Marine	
Nitrate (test strip)	Marine	
Nitrite (test strip)	Marine	

Notes:

ENVIRONMENTAL OBSERVATIONS

Recorder's name: _____ Date: _____

Sky conditions	1 - sunny	2 - mostly sunny	3 - partly sunny	4 - mostly cloudy	5 - cloudy
Amount of cloud cover	no clouds	1/8 to 2/8	3/8 to 1/2	5/8 to 7/8	total coverage

Current precipitation	1 - none	2 - drizzle	3 - rain	4 - snow	5 - sleet	6 - fog	7 - heavy rain
Barometric Pressure (kPa)							

Water colour ***(see guide)***	1 - blue	2 - blue-green	3 - light green	4 - dark green	5 - green-brown	6 - brown	7 - grey
Surface appearance:	1 - clear	2 - scum	3 - foam	4 - debris	5 - sheen	6 - other	7 - aquatic plants/algae
Water odour:	1 - none	2 - oil	3 - acrid	4 - sewage	5 - rotten egg	6 - fishy	7 - musky

Turbidity	1 - clear	2 - slightly cloudy	3 - cloudy	4 - opaque (solid)	
Wave conditions	1 - calm	2 - ripples	3 - waves	4 - white caps	
Wave frequency	1 - calm	2 - normal	3 - rough	**Wave height** ***(estimate meters)***	_____ m
The longshore current direction (direction of current parallel to shoreline) and speed			Direction (S of 180°) _____		

	Speed (cm/sec) ______________________________

Sources of Discharge: River, Pond(s), Wetland(s), Outfall(s), Other(s)					
Type					
Name of Source					
Amount	☐ Gushing ☐ Steady stream ☐ Trickle	☐ Gushing ☐ Steady stream ☐ Trickle	☐ Gushing ☐ Steady stream ☐ Trickle	☐ Gushing ☐ Steady stream ☐ Trickle	☐ Gushing ☐ Steady stream ☐ Trickle
Characteristics	☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Floatables ☐ Oily Sheen	☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Floatables ☐ Oily Sheen	☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Floatables ☐ Oily Sheen	☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Floatables ☐ Oily Sheen	☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Floatables ☐ Oily
If other, describe the feature identified					

Wildlife - count at survey start time			People and water users
Ducks	Ducklings	Gulls	**(type + quantity)** *e.g., number of people sailing, canoeing, kayaking, stand-up paddleboarding, swimming, etc.* *Swimmers:* *Boaters:* *Paddlers:* *Kayakers/Canoers:* *Dogs:* *Walkers:* *Fishers:*
Canada Geese	Goslings	Cormorants	
Terns	Swallows	Herons	
Carp	Redwing Blackbirds	**Other:** (species + quantity)	
Minnows	Fish (unidentified)		

Frogs	Turtles		**Tallying method: 𝍸 = *5***
DEAD/ INJURED ANIMAL: ENTANGLED? (Y/N):			

Floatables (Tally): 𝍸 = *5*							
Food & drink related:	plastic bags	plastic bottles	wrappers/ packaging	cups/plates/ cutlery	straws	water bottle lids	OTHER
Sewage/ Medical waste:	condoms	tampons and applicators	dental hygiene	feminine pads	wipes	syringes	OTHER

Litter/ Other	clothing/ shoes	balloons	styrofoam (< 1 cm)	styrofoam (> 1 cm)	paper	cigarette butts	OTHER
Building material				**Fishing Gear:** line, lures, nets, bate containers			

Appendix C : Environmental Health and Safety Survey

RECREATIONAL SWIMMING AREA ENVIRONMENTAL HEALTH AND SAFETY SURVEY (EHSS) CHECKLIST

Identification

Beach Name:
Address:
Responsible Authority:
Tel.:
E-mail:
Person(s) Conducting Survey:

Date:
Time:

Background Information

Water Body Type:

Dimensions of Beach	Length (m):	Width (m):	
Dimensions of Swimming Area	Length (m):	Width (m):	Area:
Number of Sampling Sites:			
Water Temperature High (°C):	Water Temperature Low (°C):		Average (°C):
Prevailing Winds Direction:	Avg. Speed (km/h):		
Prevailing Currents Direction:	Avg. Speed (km/h):		
Seasonal Rainfall Total (mm):	24-h High (mm):		
Wave Height Average (m):	Range (m):		

Surrounding Land Uses (check all that apply):

Urban □	Rural □	Residential □	Forest □	Marsh/Swamp □	Landfill □
River/Stream/Ditch: □	Field □		Hills/Uplands □	Harbour □	Picnic area □

Agricultural (specify): _____ □ Commercial (specify): _____ □

Industrial (specify): _____ □ Other: _____ □

A. Microbiological Hazards Assessment

Potential Sources of Faecal Contamination

Municipal Sewage Discharges □ Stormwater Drains/Discharges □ Septic Waste Systems □

Wastes from Animal Feeding Operations □ Combined Sewer Overflows (CSOs) □

Other Discharges Containing Faecal Wastes (List): _____ □ _____ □

Other Sewage Collection/Disposal/Treatment Systems (List): _____ □ _____ □

Stormwater Runoff from:

Agricultural Areas □ Areas Receiving Sewage Sludge □ Beach and Surrounding Facilities (e.g., parking) □

Other: _____ □ Other: _____ □

Other Environmental Sources:

Discharging Rivers/Streams/Creeks □

Birds (e.g., gulls, ducks, geese, other) □ (#'s: None Low Med High [circle one])

Other wild animals □ (#'s: None Low Med High [circle one])

Pets □ (#'s: None Low Med High [circle one])

Swimmers □ (#'s: None Low Med High [circle one])

Other: _____ □ (#'s: None Low Med High [circle one])

Items for Consideration during the Microbiological Hazards Risk Assessment:

- Proximity of potential contamination sources to the swimming area
- Potential for contamination sources to have an impact on the swimming area (including an indication of their risk priority: Low, Medium, High)
- Evaluation of water quality according to historical microbiological data (e.g., frequency of exceedances of the guideline values for the recommended indicators of faecal contamination [e.g., continuous/periodic/sporadic])
- Discharges: Assessment of such factors as volume, flow rate, treatment type, applicable indicator standards, periodicity (continuous, sporadic), and predictability

- Effects of rainfall: Levels triggering contamination events and typical event duration
- Assessment of swimming area circulation: Effect of onshore winds, tides, currents, flow patterns in transporting faecal contamination to and entrapping it within the swimming area
- Animals and birds: Assessment of their types, numbers, and droppings
- Impact of swimmers on water quality: numbers, ages
- Assessment of potential barriers: Barrier types and points at which they may be applied to reduce impact of the contamination source and/or swimmer exposure

B. Chemical Hazards:

Potential Sources of Chemical Contamination

Commercial/Industrial Discharges □ Motorized Watercraft □

Other: _____ □ Other: _____ □

Stormwater Runoff From:

Urban areas □ Areas subject to pesticide application □ Areas subject to fertilizer application □

Other: _____ □

Items for consideration during the Chemical Hazards Risk Assessment:

- Proximity of potential contamination sources to the bathing area

- Potential for contamination sources to have an impact on the swimming area (including an indication of their risk priority: Low, Medium, High)
- Discharges: Assessment of such factors as volume, flow rate, treatment type, periodicity (continuous, sporadic), and predictability
- Effects of rainfall: Levels triggering contamination events and typical event duration
- Assessment of swimming area circulation: Effect of onshore winds, tides, currents, flow patterns in potentially transporting chemical contamination to and entrapping it within the swimming area. Motorized watercraft: Assessment of their types and numbers.
- Assessment of potential barriers: Barrier types and points at which they may be applied to reduce impact of the contamination source and/or swimmer exposure. Chemical hazards

C. Other Biological Hazards

Other Biological Hazards Known to Affect the Recreational Water Area (presence may be continuous, seasonal, or sporadic)

Cyanobacterial Blooms □ Schistosomes (Swimmer's Itch) □ Large Numbers of Aquatic Plants □

Other (specify): _____ □ Other (specify): _____ □

Items for Consideration during the Biological Hazards Risk Assessment:

- Seasonal nature of the hazard: continuous, annual, sporadic
- Presence of contributing factors (as applicable): water conditions, local geography, temperatures, nutrient levels, presence of appropriate host species
- Assessment of potential barriers to control hazard and/or reduce human exposure in areas/during times of increased risk

Physical Hazards and Aesthetic Considerations

Subsurface Hazards:

Steep Slopes or Drop-offs □ Depths greater than 4.5 m □ Large Rocks □ Slippery or Uneven Bottom □

Other: _____ □ Other: _____ □

Water Conditions:

Strong Currents or Rip Tides □ Undertows □

Other:

Litter on Beach □ (None Low Med High [circle one])

Floating Debris □ (None Low Med High [circle one])

Broken Glass or Other Sharp Objects □ (None Low Med High [circle one])

Medical Wastes □ (None Low Med High [circle one])

Seaweed/Algae on Beach □ (None Low Med High [circle one])

Vehicles Permitted on Beach or Near Bathing Area:

Automobiles Y / N

Boats/Watercraft Y / N Specify:

Items for consideration during the Resulting Risk Assessment:

- Assessment of the physical characteristics of the beach and their potential impacts on safe enjoyable use of the area. Includes evaluation of physical layout (geography, topography), composition of shoreline and bottom material, influence of existing structures.

- Assessment of potential risks posed by specific hazards/factors in causing injury or illness or otherwise interfering with the enjoyable use of the area.

- Shoreline and water free from obstructions and of sufficient clarity to permit viewing of persons who may be in distress.
- Assessment of the nature and origin of litter and floating debris.
- Applicable physical and aesthetic parameters (pH, temperature, turbidity, colour, clarity, litter) in agreement with recommendations given in the Guidelines for Canadian Recreational Water Quality.
- Assessment of potential barriers to control hazard and/or reduce human exposure in areas/during times of increased risk.

Facilities and Safety Provisions

Facilities:

Toilets #: ___ □ Showers #: ____ □ Drinking Water Fountains #: __ □ Litter Bins #: ____ □ Access for Persons with Disabilities □
Other: #: ____ □ Other #: ____ □

Safety Provisions:

Lifeguard Stations #: ____ □ Lifesaving Equipment #: ____ □ Emergency Telephone #: ____ □ First Aid Stations #: ____ □

Signs/Communication Materials:

Beach Posting/Suitability for Swimming □ Emergency Contact Information □
Other Hazards (list): _____ □ _____ □ _____ □

Formal Procedures or Reporting Mechanisms in Place to Deal with:

Municipal or Industrial Spills/Discharges/Treatment Bypasses □ Waterborne Disease Outbreaks □ Swimmer Injuries □

Items for consideration during the Resulting Risk Assessment:

- Assessment of the adequacy of facilities and safety provisions.
- Evaluation of signs and other materials for public communication: Message clear and concise, signs placed in locations highly visible to the public.

Appendix D: Lab Bench Sheet

Sample Site: _____

Sample Date: _____

Incubation Time: _____

Analysis Date: _____

Analysis Time: _____

Incubation Duration: _____

Total Coliform

Sample ID	Large Wells Total Coliform	Small Wells Total Coliform	Total Coliforms MPN/100ml	Dilution Ratio	Pass 95% Confidence

E. coli

Sample ID	Large Wells *E. coli*	Small Wells *E. coli*	*E. coli* MPN/100ml	Dilution Ratio	95% Confidence

Spatial Geomean: _____

Sampling By: _____

Analysis By: _____ **Signature:** _____

Appendix E: Cleaning SOP and Log

Introduction

This document is to be used in conjunction with the Standard Operating Procedure (SOP) for the collection and analysis of surface waters for total coliforms and *Escherichia coli* (*E. coli*) bacteria. This SOP describes the cleaning procedure of field and laboratory equipment. The purpose of the cleaning procedure is to ensure a clean environment for sample analysis and to reduce the risk of cross contamination.

All staff are responsible for understanding and implementing these procedures. If procedures are unclear, please seek the advice of the supervisor or manager.

This cleaning SOP is broken up into daily, weekly, and monthly procedures with the associated field and laboratory equipment. Record all cleaning tasks in the cleaning log with the date of completion and your initials.

General Laboratory Rules

- All users of the laboratory space are responsible for cleaning.
- Always clean up after yourself.
- Food and drink must not be in the laboratory and must not be stored in the laboratory refrigerator or freezer.
- Always wash your hands before and after analysis and cleaning.
- All surfaces and equipment should be viewed as potentially contaminated.
- Laboratory floors and tables/benches should be kept free of clutter.
- Materials (gloves, pipette tips, cleaning solutions, deionized water, Colilert, etc) are to be stored in cupboards.
- Boxes are to be labelled and should be in view.

Materials and Equipment

- Paper towel
- Laboratory gloves
- Laboratory coat

- Broom
- Mop and bucket
- Soft cloth
- Cleaning sponge
- Access to a sink
- Cleaning solutions: mild detergent, diluted bleach, or isopropyl alcohol

DAILY

FIELD EQUIPMENT

A. Ziplock bags/Ice packs

1. Place warm water and mild detergent into the bag and wash
2. Turn bag inside-out and wash
3. Rinse and place on drying rack to air dry

B. Secchi disc

1. Fill half of the sink with warm water
2. Add mild detergent to the water
3. Place secchi disc into the water
4. Extend the total length of the tape into the warm water
5. Pinch the tape with a wash sponge, pull the tape through the sponge
6. Wash disc and casing
7. Rinse and place on drying rack to air dry

C. Thermometers

1. Ensure that thermometer cap is screwed on tightly
2. Wash with warm water and mild detergent
3. Rinse and air dry

D. Conductivity meter

1. Remove cap
2. Wash conductivity meter and cap with warm water and mild detergent
3. Rinse and air dry

E. Water Rangers Dissolved Oxygen (DO) 25 mL sample cup

1. Ensure that the glass ampule tip is removed from the cup
2. Wash with warm water and mild detergent
3. Rinse and place on drying rack to air dry

F. Sampling cup (on the pole)

1. Remove cup from the sampling pole
2. Wash with warm water and mild detergent
3. Rinse and place on drying rack to air dry

G. Cooler

1. Remove all items from the inside of the cooler
2. Wash with warm water and mild detergent
3. Rinse and place on drying rack to air dry

Laboratory Equipment

A. Bench/lab table

1. Remove all items from the bench/lab table
2. Clean surfaces with mild detergent, diluted bleach, or isopropyl alcohol
3. Dry with paper towels

B. Sample holders

1. Place sample holders in a sink with warm water and mild detergent
2. Scrub the inside and outside with soapy water

3. Rinse and place on drying rack to air dry

C. IDEXX Quanti-Tray/2000 97-well rubber insert

1. Clean the rubber insert with warm water and mild detergent, diluted bleach, or isopropyl alcohol. The rubber insert can also be autoclaved.
2. Scrub the inside and outside with soapy water
3. Rinse and place on drying rack to air dry

WEEKLY

A. Refrigerator

1. Remove all items from the refrigerator
2. Clean surfaces (tray, bottom, top, and sides) with mild detergent, diluted bleach, or isopropyl alcohol
3. Dry with paper towels

B. Incubator

1. Turn off incubator and allow to cool (at least 30 mins)
2. Remove trays and all items (e.g., thermometer) from incubator
3. Clean surfaces (bottom, top, and sides) and trays with mild detergent, diluted bleach, or isopropyl alcohol
4. Dry with paper towels

C. Cupboards

1. Remove all items from the cupboards
2. Clean surfaces with mild detergent, diluted bleach, or isopropyl alcohol
3. Dry with paper towels

MONTHLY

A. IDEXX Quanti-Tray Sealer PLUS

Instructions can also be found on the IDEXX website.

1. Unplug the sealer and allow it to cool for at least 30 mins
2. Wear gloves
3. Open the sealer
    a. Open the access panel with a flathead screwdriver by turning the screw to the right
    b. Pull the lower access panel out and place aside
    c. Pressing on the indentation, push the door inward
    d. Lift the door upward until it clicks into place. The rollers should be exposed.
4. Clean the inside of the sealer
    a. Separate the dip tray and clean with a soft cloth or sponge with mild detergent, diluted bleach, or isopropyl alcohol
    b. Clean the rollers
    c. Clean the interior surface of the sealer
5. Clean the lower access panel
    a. From step 3b, use a soft cloth or sponge with mild detergent, diluted bleach, or isopropyl alcohol
    b. Dry with a paper towel
6. Close the sealer
    a. On the right side, lift the lever to release the door. The door should return the closed position.
    b. Place the clean lower access panel back into the sealer
    c. Close the access panel with a flathead screwdriver by turning the screw to the left
7. Outer surfaces
    a. Clean surfaces with a soft cloth or paper towel using mild detergent, diluted bleach, or isopropyl alcohol
    b. Dry with paper towels

B. Lab coats

1. Remove all items from the pocket(s) of the lab coat
2. Lab coats are NOT to be taken home to be washed

3. Autoclave lab coats if possible
4. If autoclave is unavailable, use a dry cleaning service to clean the lab coats

C. Laboratory space (floors)

1. Ensure that the space is free of items
2. Sweep
3. Using warm water and mild detergent/diluted bleach, mop the floor
4. If mop is unavailable, use paper towel with mild detergent
5. Allow floor to air dry

Cleaning Log - Daily

Item	Date	Initials										
Ziplocks/Ice packs	Sept-02	T.L										
Secchi disc	Sept-02	G.PD										
Thermometers	Sept-02	E.M										
Conductivity meter	Sept-02	C.A										
DO 25 mL cup	Sept-02	A.M										
Sampling cup	Sept-02	G.F										
Cooler	Sept-02	J.G										
Lab bench/table	Sept-02	J.R										
Sample holders	Sept-02	K.T										
IDEXX rubber insert	Sept-02	M.M										

Notes:

Date	Note

Cleaning Log - Weekly

Item	**Date**	**Initials**										
Fridge	Sept-02	T.L										
Incubator	Sept-02	G.PD										
Cupboards	Sept-02	E.M										

Notes:

Date	Note

Cleaning Log - Monthly

Item	Date	Initials										
Inside of sealer	Sept-02	T.L										
Outside of sealer	Sept-02	G.PD										
Lab coats	Sept-02	G.F										
Laboratory space	Sept-02	E.M										

Notes:

Date	Note

Appendix F: Quality Assurance/Quality Control SOP and Log

Introduction

This document is to be used in conjunction with the Standard Operating Procedures (SOP) for the collection and analysis of surface waters for total coliforms and Escherichia coli (*E. coli)* bacteria. This SOP describes the Quality Assurance/Quality Control (QA/QC) procedures and samples that are collected in the field or created in the laboratory.

Quality assurance procedures are used to ensure confidence in the quality of the sample, while procedures for quality control are used to assess the precision, accuracy, and biases of the laboratory and field sampling procedures. In addition, QA/QC samples can also be used to assess any potential contamination during the collection, transportation, processing, and analysis of the sample. The purpose of the QA/QC procedure is to ensure that any error that may be introduced during the sample collection, transportation, or analysis, is identified, measured, and properly controlled.

All staff are responsible for understanding and implementing these procedures. If procedures are unclear, please seek the advice of the supervisor or manager.

This SOP is broken up into daily, weekly, and monthly procedures with the associated field and laboratory equipment. Record all cleaning tasks in the QA/QC log with the date of completion and your initials.

General Laboratory Rules

- All users of the laboratory space are responsible for cleaning.
- Always clean up after yourself.
- Food and drink must not be in the laboratory and must not be stored in the laboratory refrigerator or freezer.
- Always wash your hands before and after analysis and cleaning.
- All surfaces and equipment are to be viewed as potentially contaminated.
- Laboratory floors and tables/benches are to be kept free of clutter.
- Materials (gloves, pipette tips, cleaning solutions, deionized water, Colilert, etc) should be stored in cupboards.
- Boxes are to be labelled and must be in view.

MATERIALS AND EQUIPMENT

- Pipette with pipette tips
- Deionized/distilled water
- Extra Whirl-Pak bags
- Soft cloth/paper towel

DAILY

FIELD

A. Temperature Blank/Indicator - Check temperature

1. To create a temperature blank, follow the steps in Table 3 (p. 14)
2. Read temperature once samples have arrived at the lab
3. Ensure that the temperature blank is a) <10℃ or b) decreasing from the initial water temperature (check field sheets).

Troubleshooting
If the temperature of the blank is similar or has increased to the initial water temperature (check field sheets), check for the following:

- Ice pack: Is it frozen/cold?
- Ice bag: Is it cold?
- Chain of command: Were the samples delivered in a timely manner?

Re-train volunteers on the SOP to ensure that the correct steps are taken for the storage and transportation of samples.

LABORATORY

A. Incubator - Check temperature

1. Turn on incubator
2. Place thermometer inside the incubator
3. Allow incubator to reach designated temperature, e.g., 35℃
4. Quickly remove thermometer and check temperature

Troubleshooting
If the desired temperature is not reached, check the following:

- On/off switch - Is the incubator on?
- Warm up duration - How long has it been?
- Check set temperature - Is the incubator set to the correct desired temperature?

B. Refrigerator - Check temperature

1. Place thermometer inside the refrigerator
2. Wait until temperature has stabilized
3. Ensure that temperature is <10℃

Troubleshooting
If the desired temperature if not reached, check the following:

- Electrical plug - Is the refrigerator on?
- Warm up duration - How long has it been?
- Refrigerator door - Is the door closed tight?

C. Ultraviolet (UV) lamp - Check for fluorescence

1. Plug in UV lamp and turn on
2. Place IDEXX comparator under the lamp
3. Ensure that the IDEXX comparator tray is fluorescing (glowing)

Troubleshooting
If the UV lamp does not turn on, check the following:

- On/off switch - Is the UV lamp on?
- IDEXX comparator - Has it expired?
- UV bulb – Is the blub damaged? (turn off the lamp before checking)

WEEKLY

FIELD SAMPLES

A. Field blank

1. Create a field blank sample by following the steps in Table 3 (p. 14)
2. Place the field blank in the cooler, proceed to the field with the cooler and field blank
3. Ensure that the field blank is placed with the samples during and after the sampling campaign
4. Upon returning from the field, process the sample by following the steps in section *2.2. Processing water samples using Quanti-Tray* Enumeration Procedure* (p. 22)
5. Analyze the field blank. There should be no positive wells for total coliforms and *E. coli*.

Troubleshooting
If there is a positive for total coliforms and/or *E. coli*,

- Deionized/distilled water - Check QA/QC log for laboratory blank. Was there contamination with deionized/distilled water?
- If no contamination is found, ensure that each cooler has a field blank for the next sampling event.
- Additional field blanks must be created to determine the source of contamination, i.e., container, field sampling, or transportation.

B. Field Duplicates

A field duplicate should be created for every 10th sample and should represent 10% of all samples taken during the field season. Results for field duplicates should fall within the 95% confidence interval. Pre-assigning field duplicates to sampling sites prior to sampling reduces biases.

1. Assign each sampling location a number.
2. For each sampling date, randomly select a number (between the numbers assigned in step 1).
3. Ensure that each sampling date is associated with a sampling location.
4. Print out the list.

Troubleshooting
If there is a positive for total coliforms and/or *E. coli*,

- Deionized/distilled water - Check QA/QC log for laboratory blank. Was there contamination with deionized/distilled water?
- If no contamination is found, ensure that each cooler has a field blank for the next sampling event.

- Additional field blanks must be created to determine the source of contamination, i.e., container, field sampling, or transportation.

Laboratory Equipment

A. Pipette - Check for accuracy

1. Attach pipette tip to pipette
2. Turn on balance, place weight boat onto the balance and press tare
3. Set pipette to the desired volume
4. Wet pipette with deionized/ distilled water
5. Withdraw the desired amount of deionized/distilled water
6. Dispense the water onto the weight boat, repeat 5 times
7. Calculate the arithmetic mean, ensure the weight of the water coincides with the density of water (1 gram per 1 mL), i.e., 10 mL of deionized/distilled water should coincide with 10 grams

Troubleshooting
If the pipette does not calibrate, check the following:
- Pipette tip - Is it securely fitted to the pipette?
- Pipette - Has it been dropped? If dropped multiple times, it could be broken.

B. Lab Blank - Check for contamination

1. Create a lab blank sample by following the steps in Table 3 (p. 14)
2. Once returned from the field, process the sample by following steps in Lab Step Two: *Processing water samples using Quanti-Tray* Enumeration Procedure* (p. 21)
3. Analyze the lab blank. There should be no positive wells for Total Coliforms and *E. coli*.

Troubleshooting
If there is a positive for total coliforms and/or *E. coli*,
- Deionized/distilled water - Check QA/QC log for laboratory blank. Was there contamination with deionized/distilled water?
- If no contamination is found, additional lab blanks must be created to determine the source of contamination, i.e., container, sample handling, or transportation.

BIMONTHLY (EVERY TWO WEEKS)

A. Conductivity meter - Calibration

It is suggested that frequent calibration is needed with more use of the conductivity meter. Prolonged storage of the conductivity meter may impact the ability of the meter to deliver accurate results. Thus, it is suggested in this SOP that the conductivity meter is to be calibrated bimonthly (every two weeks), as this would allow for the probe to be calibrated every third use (for weekly sampling).

1. Soak meter in deionized/distilled water for 30 minutes
2. Open calibration pouch to expose the calibration solution
3. Turn on the meter and place it into the calibration solution
4. Read temperature reading:
    a. If the temperature is at 25, continue to step 5
    b. If temperature is not at 25, press the down button until you see flashing values
        - Using the calibration chart, adjust the value to the correct conductivity values from the calibration chart
        - Press the “Set” button to finish
5. Rinse off the meter with deionized/distilled water
6. Place the conductivity meter back into the calibration solution. Check values against the calibration solution.

Troubleshooting
If the conductivity meter does not calibrate, check the following:

- Probe - Is it broken?
- Probe - Place probe in distilled water for 1 hour and recalibrate
- Probe - Is the probe fully submerged?
- Calibration solution - Use fresh solution
- Temperature - Ensure that the correct temperature values are used

MONTHLY

A. IDEXX Sealer PLUS - Check for seal quality

1. Pour 100 mL of tinted deionized/distilled water into a tray
2. Using the sealer, seal the tray
3. Check the quality of the seal and ensure that there is no overflow between wells or leakage

Troubleshooting
If the quality of the seal is poor, check the following:

- IDEXX sealer - Has it warmed up?
- Sample volume - Is it at 100 mL?

B. Thermometers - Check temperature

1. Using deionized/distilled water, place thermometers into individual cups
2. Pace the conductivity meter into an individual cup
3. Allow the thermometer and the conductivity meter to stabilize
4. Check consistency of temperature readings among thermometers and meters

Troubleshooting
If the thermometer does not calibrate, check the following:

- Thermometer - Are there visible cracks?
- Using the incubator, check values against the set temperature of the incubator

C. Laboratory replication - Check laboratory methods

1. Using a known positive sample for total coliform and *E. coli*, subset the sample into two individual samples (use dilution process)
2. Process the laboratory duplicate with Colilert and incubate for 24 hours
3. Analyze the laboratory duplicate
4. Using the IDEXX MPN table, ensure that both laboratory duplicates are within the confidence intervals

Troubleshooting
If replicates are not within the confidence intervals, check the following:

- Deionized/distilled water - Is it contaminated? (Check log)
- Pipette - Is it calibrated? Was it correctly used?

QUARTERLY

A. Colilert - Check for validation (positive)

1. Using known positive samples, add Colilert
2. Seal and incubate for 24 hours
3. Ensure that wells are positive for total coliforms (yellow) and *E. coli* (fluorescing/glowing)

Troubleshooting

If results indicate a negative for both total coliforms and E.coli, check the following:

- Colilert - Has the Colilert expired?
- UV bulb – Is the blub damaged? (Turn the lamp off before checking)

B. Colilert - Check for validation (negative)

1. Create sample with deionized/distilled water
2. Add Colilert, seal, and incubate for 24 hour
3. Ensure that wells are negative for total coliforms (yellow) and *E. coli* (fluorescing/glowing)

Troubleshooting

If results indicate a positive for total coliforms and *E. coli*, check the following:

- Deionized/distilled water - Is it contaminated? (Check log)
- Colilert - Has the Colilert expired?
- UV bulb – Is the blub damaged? (Turn the lamp off before checking)

C. IDEXX comparator - Check for validation

1. View if the wells of the comparator are yellow tinted
2. Using the UV lamp, check for fluorescing/glowing

Troubleshooting

If the comparator does not indicate positive for total coliforms and *E. coli* check the following:

- On/off switch - Is the UV lamp on?
- IDEXX comparator - Has it expired?

- UV bulb – Is the blub damaged? (Turn the lamp off before checking)

D. Binder Incubator - Check temperature consistency

1. Turn off the incubator and allow the incubator chamber to cool to room temperature (30 minutes)
2. Turn on the incubator and set to the desired temperature (35°C)
3. Allow the chamber of the incubator to reach the desired temperature
    a. If possible, download temperature log
    b. If not possible, place a few regular thermometers in step 2
4. Ensure that temperatures throughout the 24 hour period are 35°C +/- 0.5°C
    a. Check thermometers and compare them to the set temperature

Troubleshooting
If the incubator does not reach the desired temperature, check the following:

- On/off switch - Is the incubator on?
- Temperature - Has the desired temperature been set properly?
- Incubator doors - Is there anything preventing the inner and outer doors from closing properly?

ANNUALLY

A. External lab analysis - Check internal laboratory quality

Have an external lab process field duplicates (See Weekly B.Field duplicates) to evaluate the performance (accuracy and precision) of the laboratory by comparing results with those of other laboratories.

1. In the field, collect field duplicates for all sampling points of the site.
2. Divide samples into two identical batches.
3. Send one batch to an independent laboratory, ensuring that the laboratory receives the samples within 24 hours of the sampling time.
4. Both laboratories should use identical methods.
5. External and internal results should be within the 95% confidence interval.

Troubleshooting

If internal and external values do not fall within the 95% confidence interval, check the following:

- Training - revised field and laboratory methods
- Chain of command - revised chain of command, ensure that samples were delivered on time
- Laboratory - check for contamination within the lab space, distilled/deionized water, laboratory equipment, etc.

QA/QC Log - Daily

Item	**Date**	**Initials**										
Temperature Blank	Sept-02	T.L										
Incubator	Sept-02	G.PD										
Fridge	Sept-02	E.M										
Ultraviolet lamp	Sept-02	C.A										

Notes:

Date	Note

QA/QC Log - Weekly

Item	Date	Initials										
Field Blank	Sept-02	T.L										
Pipette	Sept-02	G.PD										
Lab Blank	Sept-02	E.M										

Notes:

Date	Note

QA/QC Log - Bimonthly

Item	Date	Initials										
Conductivity meter	Sept-02	T.L										

Notes:

Date	Note

QA/QC Log - Monthly

Item	Date	Initials										
Sealer	Sept-02	T.L										
Thermometer	Sept-02	G.PD										
Lab replicates	Sept-02	E.M										

Notes:

Date	Note

QA/QC Log - Quarterly

Item	Date	Initials										
Sealer	Sept-02	T.L										
Thermometer	Sept-02	G.PD										
Lab replicates	Sept-02	E.M										

QA/QC Log - Annually

Item	Date	Initials										
External lab analysis	Sept-02	T.L										

Notes:

Date	Note

Appendix G: IDEXX Quanti-Tray/2000 MPN Table

Appendix H: IDEXX Quanti-Tray/2000 95% Confidence Limits

Appendix I: Data Sharing information and resources

Within the database, individual field sites should be separated into individual sheets.

The first row (header row) of each sheet should be reserved for the data fields, with each column for each individual field. The header row should be centred and bolded for easier reading during data entry.

The main fields should include:

- Location identifier
- Location name
- Location coordinates (latitude and longitude)
- Sample identifier
- Collection date
- Collection time
- Method
- Substance (*E. coli*, total coliforms, enterococci or cyanobacteria)
- Substance units
- Results
- Collectors' names
- Sample kind (single grab or geometric mean)
- Sample variant (spatial or temporal geometric mean)
- Hours (number of days between the first and last input of the temporal geometric mean)
- Reference (for the geometric mean)
- Dilution ratio

Only have one piece of information per cell. Ensure that all samples, diluted samples, and field duplicate samples are input into the database with the proper labels, as outlined in Table 1. Consistency in the values entered into the database is key, meaning the format of the entered values should match exactly those from the instruction document, i.e., dates should follow YYYY-MM-DD, times should be HH:MM:SS (24-hour format), and site location (latitude and longitude) should be in decimal degrees (DD.DDDD). This promotes the reusability of the data. Newer data should be placed at the top nearest to the header row, with older entries below it.

Sample Datasheet for recording

station Id	location Id	Location Name	latitude	longitude	sample Id	collection Date	collection Time	method	substance	units	result	advisory	collector	SampleKind	sample Variant	hours	reference	WaterTemperature	depth	secchi Depth
6	6A	Marina_Four	43.6388090	-79.3843384	ID18 - 6A-38	2018-09-27	09:52:00	9223B_colilert	ecoli	MPN	146.7		Elise	single		24	ID18-6GMT-38	14	1.53	1.40
6	6B	Marina_Four	43.6388090	-79.3843384	ID18 - 6A-38-DUP	2018-09-27	09:55:00	9223B_colilert	ecoli	MPN	127.4		Elise	single		24	ID18-6GMT-38	14	1.53	1.40
6	6C	Marina_Four	43.6384066	-79.3843933	ID18 - 6B -38	2018-09-27	10:00:00	9223B_colilert	ecoli	MPN	44.1		Elise	single		24	ID18-6GMT-38	14	1.61	1.54
6	6D	Marina_Four	43.6380505	-79.3843397	ID18 - 6C-38	2018-09-27	10:07:00	9223B_colilert	ecoli	MPN	60.2		Elise	single		24	ID18-6GMT-38	14	1.43	1.33
6	6D-DUP	Marina_Four	43.6380505	-79.3843397	ID18 - 6D-38	2018-09-27	10:10:00	9223B_colilert	ecoli	MPN	57.3		Elise	single		24	ID18-6GMT-38	14	1.77	1.45
6	6E	Marina_Four	43.6377589	-79.3843553	ID18-6E-38	2018-09-27	10:17:00	9223B_colilert	ecoli	MPN	73.3		Elise	single		24	ID18-6GMT-38	14	1.50	1.23
6	6F	Marina_Four	43.6388090	-79.3843384	ID18-6F-38	2018-09-27	09:46:00	9223B_colilert	ecoli	MPN	119.8		Elise	single		24	ID18-6GMT-38	14	1.30	1.12
6	6	Marina_Four	43.6377402	-79.3849471	ID18-6GMT-38	2018-09-27	10:00:55	9223B_colilert	ecoli	MPN	77.81		Elise	geomean	spatial	24	ID18 - 6A-38, ID18 - 6A-38, ID18 - 6B - 38, ID18 - 6C-38, ID18-6E-38, ID18-6F-38			

Frequently Asked Questions

Are Whirl-Paks sterile?
All Whirl-Pak bags (118 mL) undergo sterilization after manufacturing.

Will rainwater affect the results?

Rainwater has relatively fewer impurities and microbial communities, thus it is reasonable to believe that sampling during rain will not affect the results.

Why must the sampling end (of the sampling pole) be rinsed three times?

This is to reduce the risk of cross contamination between sampling locations.

What to do with Overfilled or unfilled Whirl-Paks or bottles?

If a Whirl-Pak or Whirl-Pak/bottle is overfilled, carefully pour out the contents until they reach 100 mL line (bottle) or between the 100 mL-118 mL (Whirl-Pak). If a Whirl-Pak/bottle is underfilled, pour out the contents away from the sampling location and resample. Do not resample the location with existing water in the container.

Why should there be a headspace (air space) when sealing a Whirl-Pak?

Having a headspace will help prevent spilling during transportation.

What if temperature blanks record outside of the 0° C to 10 °C range upon receipt at the lab?
Samples should be stored and transported in a cooler, at between 0°C and 10°C (preferably 4°C), and kept at this temperature until processing. There are two main reasons why sample temperatures might not fall within the 0°C and 10°C range.

Above 10 °C
Samples must be kept in a cooler, with wet ice or ice packs, to keep them cold. There are several potential reasons that a cooler did not keep the samples cold enough:

- The cooler was left open between samples and therefore the temperature of 0°C to 10°C was not maintained

- Not enough ice was used to keep the cooler at a temperature of 0°C to 10°C
- Outside temperatures were such that the cooler struggled to keep samples cool

Beach water can be above 10°C and water at recreational sites is often above 20°C at sample locations. One reason sample temperatures do not read 10°C and under is that they simply did not have enough time to cool down between collection and arrival to the lab for processing. Samples taken from a water body reading 20°C or above can take anywhere from 1-2 hours to cool down to <10C. If a temp blank reads above 10°C, check what the average water temperature was at the collection time to determine whether the temp blank had enough time to get cold. If it is determined that the temp blank did not have enough time to cool down before processing, note the temperature and include a note about the water temperature at the site and time elapsed between collection and delivery. Proceed with sample processing.

What to do with empty and partially full wells (Quanti-Trays)?

Any well that has liquid present is used for the interpretation of the results. Partly full/empty wells could be the result of inadequate sample volume being added to the Quanti-tray with the reagent. The reagent powder accounts for approximately 3 mL. The large well on top is counted as one well, although it is designed to hold close to 5 mL.

All microbiological quantitative methods, such as MF, MTF, or Quanti-tray, are based on the Poisson distribution. It is not an exact number, but represents that the value obtained lies between two intervals with 95% confidence.

For example:

A Quanti-Tray/2000 with 29 positive large wells and 48 positive small wells. Using the MPN table, this would result in a MPN value of 130.8. This value with 95% confidence, can be as high as 155.9 and as low as 108.6.

If there were 28 positive large wells and 48 positive small wells (a difference of 1 well from the above), the MPN value would be 125.6 with a 95% confidence interval of 104.2 and 149.8.

If there were two empty wells—a very rare occurrence unless the volume of the sample is below 98 mL—the result obtained for 27 positive large wells and 48 positive small wells, is 120.7 with a 95% confidence interval of 100.2 and 144.2.

Using the same logic as indicated above, there is no significant difference between the 3 examples, since all values are within the confidence interval.

What to do when duplicate samples do not fall within the 95% confidence interval

There are several reasons why results for duplicate samples fail to fall within the 95% confidence interval. When encountering this issue, review the following:

- Review field sheets to determine the labelling of the containers: Perhaps the IDs were switched, the sample was incorrectly labelled, etc.
- Weather conditions, specifically wave action: During wavy conditions sediment resuspension may cause large variations between duplicate samples.
- Contamination: Check field blanks and the condition of the cooler for signs of contamination. Review the cleaning log if necessary.
- Review the lab bench sheet: Ensure that the processing, incubation, and analysis times were consistent between the duplicate samples.
- Training: Review the SOP with the sampler, as new volunteers may not have the necessary sampling consistency.

Incubator Temperature : What is the protocol for samples that were not kept at 35°C?

IDEXX has been able to validate results for 24-hour Colilert at incubation temperatures of 36°C +/- 2, i.e., as low as 34°C or as high as 38°C. The US EPA mandates incubation at 35°C +/- 0.5°C; however, Colilert evaluations done in the UK were incubated and validated at 37°C. Ultimately, if these samples are for regulatory purposes, we recommend following appropriate local regulatory procedures.
If the facility has a water bath that is able to maintain temperature, it is possible to use that bath to keep samples at 35°C for the incubation period in lieu of a working incubator.

What if a well/s is/are positive for E.coli but not Total Coliforms?

Wells that show fluorescence without yellow colour are a valid negative for both total coliforms and *E. coli*. In order for an *E. coli* positive result, both colour and fluorescence must be present. This is something we see from time to time. A fluorescent only well is a non-specific reaction likely caused by other bacteria that are able to metabolize the MUG (4-methyl-umbelliferyl- Bd-glucoronide), which causes fluorescence. While we cannot say exactly what bacteria this is, some *Shigella* and *Salmonella* species are known to have this ability.

What should we do if there is a puncture in a sealed Quanti-Tray?

Dispose of the sample as it has been compromised.

What if a field/lab blank is positive for total coliform/E. coli?

When a field blank tests positive for total coliform/*E. coli,* it may suggest that contamination has occurred either in the field or in the lab, i.e., that the blank was incorrectly prepared. Compare the field blank with a lab blank to determine the source of the contamination and, if needed, review training for the preparation of a field blank. Prepare multiple field blanks for the next sampling campaign to determine the source of the contamination.

If a lab blank tests positive for total coliform/ *E. coli,* then it may suggest that contamination has occurred during the processing stage or that the lab blank was incorrectly prepared. Prepare multiple lab blanks to determine the source of the contamination.

What is the Colilert comparator?

The Colilert comparator is a reference item that is used to help determine if a sample well is positive or negative for total coliform or *E. coli*.

How do I use the Colilert comparator?

After incubation (24-28 hours), place the comparator near the sample Quanti-Tray. Compare the wells of the sample Quanti-Tray to the comparator. Sample wells are positive if equal to or greater than the yellow colour or fluorescence of the comparator.

How should the comparator or Quanti-Trays be stored?

The comparator should be stored in a dark environment between 2°C and 30°C. Opened bags of Quanti-Tray or Quanti-Tray/2000 should be folded over and taped when not in use.

What should the colour and consistency of the Colilert reagent powder be?

The Colilert reagent should be white to off-white in colour, and the powder should be dry. Ensure that the snap packs are not damaged such that they allow moisture to leak in. Do not use any Colilert reagents if the snap pack is damaged or if the reagent powder is wet and has formed clumps.

Can the Colilert samples be read after 24 hours?

Yes, samples with Colilert can be read after 24 hours and before 28 hours of incubation. Samples should be discarded if the incubation duration has exceeded 28 hours.

Can the Colilert samples be read before 24 hours?

Colilert samples can be read before 24 hours but only for total coliforms and with great uncertainty. Samples should be incubated for at least 24 hours.

Why is the test limited to a 24-28-hour incubation period?

The Colilert powder contains compounds that suppress non-coliform bacteria for at least 28 hours.

How can I quantify my Colilert test results?

Colilert tests can be quantified with the Quanti-Tray, Quanti-Tray/2000, or multiply-tube method.

What are the disposal requirements for a Colilert sample?

Please check your local regulations governing waste management, particularly the biohazard and hazardous identification rules and land disposal regulations.

Is the Colilert test and the Quanti-Tray system approved by the U.S. EPA?

The Colilert test is approved by the U.S. EPA for the test of total coliforms and *E. coli* ambient (recreational) waters, surface water, ground water, and wastewater. The Quanti-Tray system (Quanti-Tray and Quanti-Tray/2000) have been approved by the U.S EPA and can be used for compliance testing.

What is the detection limit of the Colilert test?

The detection limit for the Colilert test is set at 1 bacteria (total coliform or *E. coli*) per 100 mL

What is the shelf life of the Colilert test and the Quanti-Trays?

Colilert reagents (snap pack) can be stored up to 12 months from the date of manufacture. Please check the expiry date located on the Colilert box. Both the Quanti-Tray and Quanti-Tray/2000 have a shelf life of up to 3 years from the manufacturing date.

Are the Quanti-Tray or Quanti-Tray/2000 sterile?

All trays (Quanti-Tray and Quanti-Tray/2000) have undergone sterilization by ethylene oxide gas.

Can the temperature of the sealer affect the results?

No, the temperature required for the sealing of the Quanti-Tray/Quanti-Tray/2000 will not affect the results. When sealing, the wells will not exceed a 5°C increase.

How high can I stack the Quanti-Trays or Quanti-Tray/2000s?

It is recommended that both Quanti-Trays and Quanti-Tray/2000s are to be stacked up to 10 trays high, with caution as to not block the airflow of the incubator.