Best Practices for Water Quality Monitoring

A Report Prepared for the GEF-IWEco National Sub-Project in St. Kitts and Nevis for the project entitled, "Upgrading Water Quality Monitoring Protocols in St. Kitts and Nevis".

Report submitted by Ambient Environmental Consulting Inc.

on March 1, 2022

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Scope of Work

An upgraded manual of best practices and protocols for water quality (potable, recreational and wastewater) monitoring for both islands and recommendations for training needs of laboratory personnel and review of the recommendations for the purchase of equipment as outlined in the recently completed report "Regional Laboratory Assessment" commissioned by the regional IWEco project in 2020.

1. Overview

This report was prepared following an assessment of the current water quality monitoring practices in St. Kitts and Nevis. Based upon this assessment, a series of recommendations were included in the final report on changes to these practices. A subset of these recommendations, which are summarized below, form the basis for the scope of this report that describes the recommended best practices and protocols for water quality monitoring in St. Kitts and Nevis:

- Staff need to calibrate instruments used in the field and in the laboratory on a regular basis and the dates of calibration need to be recorded in a logbook.
- Written standard operating procedures (SOPs) need to be developed for collection of water samples in the field, collection of water quality data in the field and the analysis of water quality parameters in the laboratory.
- Drinking water should continue to be primary focus for water quality monitoring in both St. Kitts and in Nevis. Critical water quality parameters include microbiological indicators, residual (free) chlorine, turbidity and salinity.
- Residual chlorine and turbidity measurements need to be conducted in the field at the time of collection of drinking water samples to avoid changes in these parameters from the time of collection to analysis in the laboratory. Appropriate field instrumentation should be purchased for this purpose.
- Nitrates should also be monitored as a critical parameter in drinking water, at least on a quarterly basis. Appropriate instrumentation and field test kits should be purchased for this monitoring effort.
- Analytical methods for indicator bacteria in drinking water samples should be switched to the IDEXX Colilert® method for analysis of total coliforms and E. *coli.* If monitoring of marine recreational waters is judged to be a priority, the Enterolert® method may also be adopted for analysis of intestinal *Enterococci.*
- If monitoring of domestic wastewater is judged to be a priority in St. Kitts, the minimum parameters for monitoring should be the levels of total suspended solids (TSS) and either biological oxygen demand (cBOD5) or chemical oxygen demand (COD).

Since the submission of the report on current water quality monitoring practices in St. Kitts and Nevis, additional information was provided to the consultants that has a bearing on the recommendations for best practices in water quality monitoring in St. Kitts and in Nevis:

- Funding has been secured to purchase and install multi-parameter water quality sondes at 20-25 of the wellheads supplying water for treatment and distribution in St. Kitts and at all the wellheads supplying water for treatment and distribution in Nevis. These probes have the capacity for unattended continuous monitoring and on-board data logging. The preferred model for acquisition, the Ott Hydrolab HL4 has the capacity to monitor for temperature, oxidation reduction potential (ORP), depth and up to four other parameters, which could include pH, conductivity, turbidity, dissolved oxygen, ammonium, nitrate, chloride, chlorophyll, blue-green algae and rhodamine. The latter parameter is only useful when using rhodamine dye as a tracer for hydrological studies. **The four additional parameters recommended for continuous monitoring at the wellhead are: pH, turbidity, nitrate, and either conductivity or chloride.**
- The Water Services Department at St. Kitts has been monitoring water at the wellheads on approximately a monthly basis since 2015 for total dissolved solids (TDS) and more recently (since 2019), also for salinity. These data indicate that the water supplied at the Conaree and Ponds 2 wellheads are at the lower limits of palatability in terms of TDS and salinity, and the water supplied at the Factory wellhead is marginal in terms of water quality. **These data reinforce the previous observation that some wells in St. Kitts are showing signs of saltwater intrusion and deteriorating water quality.**
- The Water Services Department at St. Kitts currently monitors untreated and treated drinking water at the water treatment plant on a daily basis using a Thermo Orion STAR A212 instrument which measures conductivity only**. Based upon this information, we recommend that the Water Services Department develop the capacity to monitor on at least a daily basis for total and residual (free) chlorine in treated drinking water before it enters the water distribution system. A manual has been added to this report that provides an SOP for analysis of total and residual chlorine (i.e., Manual 4).**
- In Nevis, the Water Department has the capacity to monitor in the field for residual chlorine in the water collected from the distribution system using a HACH DR900 colorimeter. In the first assessment report, it was erroneously reported that residual chlorine was only monitored at Nevis with test strips. **The SOPs provided in this report for analysis of residual chlorine in drinking water now include a method using the DR900 instrument.**
- The Environmental Health laboratory in St. Kitts already has the necessary equipment to analyzed cBOD5 in wastewater, including the BOD bottles and a probe and meter for measuring dissolved oxygen. However, it is not clear whether there is currently sufficient space and access to a dedicated incubator (set at 20^{o}C) in the lab shared with the Bureau of Standards. **Notwithstanding these logistical considerations, it is recommended that cBOD5 be a priority parameter for analysis of wastewater quality in St. Kitts and that COD be considered a lesser priority.**

This first part of the report describes best practices for recording and tracking water quality data, recommendation for the acquisition of field and laboratory equipment, and suggestions for training personnel involved in water quality monitoring. The remaining part of the report documents the SOPs that are recommended for water quality monitoring in St. Kitts and in Nevis. These are divided into four manuals:

- **Manual 1:** Standard operating procedures for collecting and analyzing samples of drinking water and source waters for critical water quality parameters.

- **Manual 2**: Standard operating procedures for collecting and analyzing recreational waters for intestinal *Enterococci*.

- **Manual 3:** Standard operating procedures for collecting and analyzing samples of treated municipal wastewater.

- **Manual 4:** Standard operating procedures for collecting and analyzing samples of drinking water after disinfection for total and residual chlorine.

2. Recording and Tracking Data

The procedures recommended for recording water quality data are based on two criteria:

i) Redundancy to ensure that no water quality data are lost, or any anomalies in the data can be double checked.
ii) "Chain of custody" measures to track the progress in collecting, storing and analyzing the water samples.

To enable data tracking, unique identifier codes must be assigned to each sample. These identifiers must be used when recording all water quality data generated in the field and in the lab. Codes should include a 4-digit number that is assigned in sequence for each sampling trip within a given year. To identify the sampling site, a three letter/digit code should be assigned to each sampling location. The Environmental Health lab at St. Kitts has already assigned codes for the sampling sites (e.g., LG4 for the Port Zante standpipe). **It is recommended that the Water Department lab at Nevis develop similar identifier codes for their sampling sites**.

The SOPs described in this report specify that all data collected in the field must be recorded immediately in a field notebook. In addition, there is an option of recording data in the field in Microsoft "Forms" format using a smart phone, although this will depend on whether data connectivity is available at all sampling sites at St. Kitts and at Nevis. If the option of entering data on a smart phone in the field is feasible, an additional feature could be to mount laminated QR code posters at each sampling location to facilitate site identification and data entry.

In the laboratory, a logbook should be maintained to track when (date and time) the samples were delivered from the field to the lab and when they were stored in the refrigerator, and when the samples were analyzed. The person(s) handling the samples during each of these steps should also be recorded in the logbook. A separate logbook should be maintained to record when all instruments were calibrated and any maintenance activities.

All water quality data collected in the field and in the lab should be transcribed into a data sheet (Appendix II). All microbiological data should be transcribed into a separate data sheet (Appendix III and Appendix IV). In addition, there is an option for lab personnel to directly record data on a smart phone in the field or on a computer in the lab using the Microsoft "Forms" format. The instruments recommended in the SOPs for measuring water quality data all have the capability to record data. The lab manager should be familiar with how to retrieve these data from the instruments so that any anomalies in recording of the data can be double checked.

The current procedure for handling data entered onto data sheets by technical personnel in both St. Kitts and in Nevis is for the lab manager to transcribe the recorded data into an Excel spreadsheet. However, Microsoft Forms can be used to set up direct entry of the data by technical personnel using a smart phone or a lab computer. Data entered in the field or in the laboratory into the forms are automatically integrated into an Excel spreadsheet.

The lab manager should be familiar with data base management tools that can be used to organize data so that trends in water quality data can be monitored. For instance, Excel worksheets can be integrated into Microsoft Access to manage or merge multiple data bases. The lab manager should be able to evaluate the data for quality control/quality assurance purposes. A data validation step could be included in Excel spreadsheets to catch mistakes in data entry. To enable management of water quality data, all samples must be tracked at each step in the process of collection and analysis using the unique identifier codes assigned to each sample. The Microsoft product, Power Automate® can be used to set up workflows for the data that are entered, automated sharing of data with stakeholders. Data that exceed regulatory guidelines can also be automatically flagged for additional scrutiny. A site license for this system can be purchased for as little as $15 USD per month for each of the lab managers at St. Kitts and at Nevis.

Note that the SOPs in this report indicate "trigger" values to advise technical personnel when they should alert the lab manager or a supervisor to unacceptable quality of water. In the case of unacceptable levels of total coliforms and E. *coli* in samples of drinking water collected from the water distribution system, the lab manager should take immediate action to collect additional samples for analysis and/or to alert the public health officer to the threat to human health. The public health officer should contact the appropriate agency to recommend increasing the dose or the frequency of chlorine disinfection, and if necessary, to issue a 'boil water' advisory to the public. The SOP for analysis of nitrate also indicates a trigger value to advise lab personnel when they should alert the lab manager to unacceptably high levels of nitrate in drinking water. In this case, the lab manager should take action to collect additional samples for analysis and should also alert the public health officer to a potential threat to human health.

3. Recommendations for Acquisition of Field and Laboratory Equipment

Table 1 documents an analysis of equipment and instrumentation currently present or absent at the Environmental Health (EH) laboratory in St. Kitts and the Water Department (WD) laboratory in Nevis. This list was compiled by taking into account:

i) The instruments and equipment recommended for sample collection and water quality analysis using the standard operating procedures described later in this report.

ii) The currently available instruments and equipment in the Environmental Health laboratory in St. Kitts and the Water Department laboratory in Nevis.
iii) The instruments documented and recommended for acquisition in the IWEco (2021) report entitled, "Laboratory Assessment Report for Caribbean Countries Participating in the GEF-IWEco Project".
iv) The instruments, equipment and facilities requested by personnel from the Environmental Health laboratory in St. Kitts and the Water Department laboratory in Nevis, as documented in the previous report from this consultancy on "Water Quality Monitoring Practices in St. Kitts and Nevis".

It is beyond the scope of this consultancy to make recommendations on upgrades to the laboratories at St. Kitts and in Nevis. However, the consultants are particularly concerned about the lack of safety equipment (i.e., emergency shower, eyewash station) and a fume hood in the lab in Nevis, and the lack of procedures for disposing of potentially hazardous liquid waste in both laboratories (i.e., liquid wastes are currently flushed down the drain). While staff with the Environmental Health lab in St. Kitts benefit from access to equipment owned and maintained by the Bureau of Standards (i.e., deionized water generator, fume hood, autoclave, incubator, safety equipment), the consultants are concerned about the current lack of space for carrying out lab procedures and recording and managing data.

Table 1: Equipment and instruments needed for sample collection and analysis of water quality parameters, as described in the SOPs in this report and their status (absent or present) in the Environmental Health laboratory in St. Kitts and the Water Department laboratory in Nevis.

Recommended Parameter	Equipment/Instrument	EH lab St. Kitts	WD lab Nevis
Sample collection	Sampling pole	Absent	Absent
Sample storage	Dedicated refrigerator/freezer	Absent	Absent
Data entry	Laptop computer	Absent	Absent
Reagent preparation	Deionized water generator	Present	Absent
Free chlorine[a]	HACH Pocket Colorimeter II **or** HACH DR900 Colorimeter	Present Absent	Absent Present
Total coliforms[a]	IDEXX test system[b]	Absent	Absent
E. *coli*[a]	IDEXX test system[b]	Absent	Absent
Turbidity[a]	HACH 2100Q turbidity meter	Absent	Present
Nitrate[a]	HACH DR300 Pocket Colorimeter (nitrate)	Absent	Absent
Salinity[a]	HACH Pocket Pro+ Multi-2	Absent	Present
Water temperature	HACH Pocket Pro+ Multi-2	Absent	Present
pH	HACH Pocket Pro+ Multi-2	Absent	Present
Conductivity	HACH Pocket Pro+ Multi-2	Absent	Present
Salinity	HACH Pocket Pro+ Multi-2	Absent	Present
TDS	HACH Pocket Pro+ Multi-2	Absent	Present

a) "Critical" water quality parameters that are essential for water quality monitoring.
b) Quanti-Tray sealer, UV-lamp cabinet, consumables.

From this analysis, the following equipment and instruments are recommended for acquisition:

- Sampling poles – Both labs
- Dedicated refrigerator/freezer – Both labs
- Laptop computers – Both labs
- Deionized water generator – WD lab, Nevis
- IDEXX test system equipment – Both labs
- HACH 2100Q turbidity meter (field) – EH lab, St. Kitts
- HACH DR300 pocket colorimeter for nitrate (field or lab) – Both labs
- HACH Pocket Pro+ Multi-2 meter (field) – EH lab, St. Kitts

The purchase of low-cost items such as thermometers and a hygrometer requested by the Nevis WD lab can certainly be accommodated. Any deviations from the IWEco recommendations and the requests for equipment from technical personnel are provided in Appendix I. Note that these recommendations are based solely on the equipment required for analysis of drinking water, source waters and recreational waters (i.e., Manual 1 and Manual 2). The recommended equipment for analysis of treated municipal wastewater are described separately in Manual 3.

Finally, it is recommended that a HACH DR300 Pocket Colorimeter (chlorine) be purchased for monitoring of total and residual (free) chlorine by the Water Services Department at St. Kitts in the disinfected drinking water prior to entering the water distribution system. SOPs for total and residual chlorine are provided in Manual 4.

The IWEco report indicated that a DR3900 spectrophotometer is available in the Environmental Health (EH) laboratory in St. Kitts but it needs service. It would be worthwhile to have it serviced if analysis of COD in wastewater samples (see Manual 3) is judged to be a priority. Lab personnel would need training on its operation and maintenance

Recommendations for Training of Personnel

The consultants feel that the SOPs provided in the present report for collecting and analyzing water samples, plus reviews of training videos available on YouTube will provide the technical personnel with sufficient skills to assist in a monitoring programme for the quality of drinking water and source waters. **For analysis of wastewater, a training programme for technical staff will be necessary.** The consultants also agree with the recommendations for additional training that were documented in the IWEco (2021) report entitled, "Laboratory Assessment Report for Caribbean Countries Participating in the GEF-IWEco Project". These recommendations and others identified by the consultants include training in:

- ISO 17025 requirements and implementation for inventory of equipment and chemicals.
- Health and safety procedures, including the Workplace Hazardous Materials Information System (WHIMS).
- Calibration, verification of accuracy and in-house maintenance of field and laboratory equipment, with associated record keeping.
- Record keeping and statistical analysis of analytical results for quality control/quality assurance purposes and to monitor temporal and spatial trends in the data.
- Development of on-line systems for data management and sharing of data with stakeholders.

MANUAL 1

Standard Operating Procedures for Collecting and Analyzing Samples of Drinking Water and Source Waters for Critical Water Quality Parameters

1 Preparing for sample collection

Before going out into the field to collect water samples, calibrate all field meters according to the manufacturer's instructions and make sure that the meters contain fully charged batteries. Prepare plastic sample bottles and confirm the four-digit sample identifier number to be applied to all samples during the sampling trip. Assemble a field kit that includes:

- Coolers with cold packs to store the samples in the field.
- An appropriate number of glass or plastic sample bottles (250 or 500 mL) with labels attached.
    - For collection of samples for microbiological analysis, the sample bottles need to be sterile, and if the samples for microbiological analysis are to be collected from the drinking water distribution system, they need to contain sodium thiosulphate solution to neutralize residual chlorine (see below).
    - For analysis of other water quality parameters, sample bottles do not have to be sterile, but prior to sample collection, they must be thoroughly cleaned with soap and water, then rinsed with deionized (or distilled) water, dried and labelled.
    - If additional water quality parameters are to be monitored (e.g., arsenic), take an appropriate number of labelled sample bottles provided by the laboratory that will do the analysis.
- Bleach solution (see below) and a roll of paper towels.
- Field colourimeter for analysis of residual chlorine.
- Field turbidity meter.
- Field salinity meter. The recommended field meter can also be used to monitor for temperature, pH, conductivity and TDS.
- Containers for collecting liquid and solid wastes.
- Waterproof field notebook, pens and labelling markers.
- Sanitary wipes or hand sanitizer.
- Smart phone for recording field data (optional).

Sterilized bottles with sodium thiosulphate: *Pre-sterilized sample bottles containing thiosulphate are available from commercial suppliers. However, if sample bottles are to be re-used, before each sampling trip prepare a fresh 3% thiosulphate solution by dissolving 3 grams of anhydrous sodium thiosulphate (*$Na_2S_2O_3$) in *100 mL of deionized (or distilled) water. Then, transfer 0.2 mL of solution into each 250 mL bottle or 0.4 mL of solution into each 500 mL bottle. Loosely fit the cap on each bottle (do not tighten) and sterilize in an autoclave at 120°C with 15 psi pressure for 20 minutes. Remove the sample bottles from the autoclave, allow to cool, tighten the cap on each bottle and apply a label.*

Bleach solution: *Make a fresh bleach solution before each sampling trip by adding 15 mL of household bleach to 100 mL of tap water. Carry in a plastic bottle to the field.*

2. Collecting water samples

Drinking water

- The person collecting the samples must clean their hands with sanitary wipes or hand sanitizer before collecting each sample.
- Prior to collecting the sample, disinfect the faucet with a paper towel soaked in a small volume of the bleach solution.
- Let water run through the faucet for 3 minutes before collecting the sample.
- When collecting the sample, reduce the flow if possible and avoid overfilling or splashing the outside of the bottle.
- Hold the base of the sample bottle and remove the cap with your free hand, keeping your fingers pointed down to avoid contamination. Make sure you do not touch the inside of the cap or the inside of the sample bottle with your fingers.
- For microbiological analysis, collect a water sample in a sterile bottle (with thiosulphate) by filling to the ¾ mark (or to a fill line, if provided). Replace the cap without touching the inside and make sure the cap is tight. Label the bottle with a sample identifier (see below), the date and time of sample collection, the type of sample (i.e., bacteria) and the initials of the person who collected the sample. Place the sample bottles as soon as possible in a cooler with ice packs.
- For analysis of nitrates, collect an additional sample in a labelled non-sterile sample bottle, as described above and then label the bottle with a sample identifier, the date and time of sample collection, the type of sample (i.e., nitrate) and the initials of the person who collected the sample. Place the sample bottles as soon as possible in a cooler with ice packs (see note below).
- For samples to be analyzed for additional water quality parameters, collect a sample as described above using a bottle provided by the external laboratory, then label. Place the sample bottle as soon as possible in a cooler with ice packs.
- Collect an additional sample in a non-sterile bottle for analysis of other parameters that are to be analyzed in the field (i.e., free chlorine, turbidity, salinity, pH, TDS, water temperature), no label is required. Collect the field data as soon as possible after collection.

Sample identifier: *To track water quality data, a unique identifier must be assigned to each sample. This identifier should include a 4-digit number that is assigned in sequence for each sampling trip within a given year (e.g., 2201 for first trip in 2022; 2202 for second trip in 2022, etc.). A three letter/digit code (e.g., CA1) is used to identify the sampling site. These identifiers should be used when recording all water quality data generated in the field and in the lab.*

Preservation of samples for nitrate analysis: *If a delay of more than 48 hours is anticipated before the samples can be analyzed for nitrate, then the samples must be preserved when returned to the lab by acidifying to pH 2 with dilute sulfuric acid. Otherwise, transport in a cooler and refrigeration in the lab prior to analysis is adequate for sample preservation.*

Source waters

Groundwater:
Samples of groundwater collected at the wellhead can be collected as described previously for sampling drinking water in the distribution system. If water is pumped continuously at the wellhead, then samples can be collected as described previously. However, if water is pumped intermittently at the wellhead, then the pump should be run for several minutes prior to sample collection until a constant level of turbidity is reached. Once the water samples are collected, label the bottle with a sample identifier, the date and time of sample collection, the type of sample (i.e., bacteria, nitrate) and the initials of the person who collected the sample. Store the samples in a cooler or collect water quality data in the field as described previously for drinking water.

Surface waters:
Samples from small springs can be collected by hand as described previously for the collection of samples of drinking water. In larger streams, it is recommended that an extendable sampling pole with a holder for securing a sample bottle be used for collection of surface waters (Figure 1). Safety concerns should be paramount when there are high flows in streams during or after rain events. Do not collect a sample if you are concerned about your safety, or if there is a high risk of losing the sampling pole or sample bottle because of high flows. Always sample surface waters with a partner.
Note that this type of sampling pole can also be used for collection of samples from water cisterns, surface waters at recreational beaches and the effluents from wastewater treatment facilities.

The following procedures are to be used by two persons for collecting surface waters with a sampling pole:

- If samples are to be analyzed for microbiological parameters, the persons collecting the samples must clean their hands with sanitary wipes or hand sanitizer before collecting the samples.
- Extend the telescoping sampling pole enough so that you can safely collect the sample from the stream bank at a point as close as possible to the center of the stream. The sampling pole should always be extended horizontally, never straight up.
- Rinse the sampling end of the extending sampling pole in the stream by dipping it in the water. Take care not to disturb bottom sediment when rinsing.
- Back up from the stream bank with the pole (keeping it horizontal) until the sampling end of the pole is over land and can be safely accessed by your sampling partner.
- Your sampling partner then must secure sample bottle in the holder for a snug fit around the bottle.
- Your sampling partner then must remove the lid of the sample bottle without touching the inside of the bottle or the lid. Your sampling partners should place the lid face up on a stable surface until the bottle is retrieved and ready to be sealed.
- Extend the sampling pole horizontally out in front of you and lower the sampling end down into the stream so that the mouth of the bottle is facing upstream, then gently submerge the bottle to a depth of approximately 5-10 cm below the surface. If there is

adequate depth, submerge the sample bottle at a 45^{o} angle to the flow. If the water is very shallow, submerge the bottle parallel to the flow.

- Once the bottle is filled (after bubbling has stopped), carefully lift the sampling pole from the water by raising the sampling end until it is extended horizontally out in front of you.
- Back up from the stream bank with the pole still extended in front of you. Your sampling partner must grab the sampling end of the pole (taking care not to contact the sampling bottle) and help bring the bottle back to the shore. Do not raise the pole in a vertical position.
- Check whether the bottle contains an adequate volume of water, as the bottle should be at least ¾ full. If the bottle does not contain the required volume, dump the sample and repeat the sampling procedure. A new bottle is required for microbiological samples. For samples for all other water quality parameters, the bottle can be emptied and reused.
- Your sampling partners must replace the cap on the bottle without touching the inside, then loosen the holder and remove the capped sampling bottle.
- Check that the cap is tightly secured on the bottle. Label the bottle with a sample identifier, the date and time of sample collection, the type of sample (e.g., bacteria, nitrate) and the initials of the person who collected the sample.
- Repeat the sampling steps as many times as necessary to collect the required number of samples. This includes an unlabelled sample for collection of data in the field (e.g., turbidity, salinity, temperature, pH, TDS, etc.).
- Store samples in a cooler and collect water quality data in the field as described previously for drinking water samples.

3. Analyzing water samples

The water quality parameters that were recommended as critical for monitoring in drinking water and in source waters include:

- Residual (free) chlorine for samples collected from the water distribution system
- Microbiological indicators (i.e., total coliforms, E. *coli*)
- Turbidity
- Salinity
- Nitrate

3.1 Residual chlorine

The SOP described below is based on use of either the **Hach Pocket Colorimeter II** (available at the EH lab in St. Kitts) or the **Hach DR900 Colorimeter** (available at the WD lab in Nevis) for testing of residual (free) chlorine in the field. Ensure that fully charged batteries are installed in the meter before taking the colourimeter into the field. The method requires addition of DPD free chlorine reagent (Product # 2105569-CA) to the water sample prior to analysis.
There is an instructional video for the Pocket Colorimeter II on YouTube: https://www.youtube.com/watch?v=G2wSu6mN9Eo.
There is an instructional video for the DR900 Colorimeter on YouTube:
https://www.youtube.com/watch?v=T3JV9VejLUQ

- Residual chlorine must be measured in the field **as soon as possible** after collection of the sample from the drinking water distribution system; ideally within 1 minute of collection of the sample.
- Pocket Colorimeter II: Turn on the meter with the power button and push the menu button. Using the check mark button, toggle between the high and low settings for analysis of residual chlorine. Select the low setting (i.e., levels of 0.02 to 2.00 mg/L) and press the menu button again to accept.
- DR900 Colorimeter: Turn on the meter with the power button. Press the "Options" (left hand) button and using the keypad, scroll to "all programs". Press the "Select" (right hand) button and scroll to "Program 80 - Free and Residual Chlorine", then press the "Start" (right hand) button.
- From the water sample collected for analysis of water quality parameters in the field, fill the sample cell with water and then dump the water. Refill the sample cell to the 10 mL line, then cap. This is the "blank" sample.
- Wipe the outside of the blank sample cell with a paper towel to remove fingerprints and any traces of liquid.
- Remove the cover from the colourimeter and place the blank sample cell in the measuring chamber. Make sure the diamond-shaped marker is facing towards the digital screen.
- Pocket Colorimeter II: With the colourimeter on a flat surface, replace the cover and press the "0" button. The digital screen should read "0.00".
- DR900 Colorimeter: With the colourimeter on a flat surface, replace the cover and press the "Zero" arrow (middle). The digital screen should read "0.00".
- Remove the blank sample cell and dump the contents.
- From the water sample collected for analysis of water quality parameters in the field, fill another sample cell with water then dump. Refill the sample cell to the 10 mL line.
- To this sample, add the contents from one packet of DPD free chlorine reagent. Cap the sample cell and shake gently. Note that the accuracy of the reading is not affected by undissolved reagent.
- Wipe the outside of the sample cell with a paper towel, then place the sample cell in the measuring chamber (marker facing the screen) and replace the cover. **This needs to be done within one minute of adding the DPD reagent to the sample.**
- Pocket Colorimeter II: With the colourimeter on a flat surface, press the button on the meter with the check mark symbol. Read the chlorine residual value on the digital screen and record the value in your field notebook.
- DR900 Colorimeter: With the colourimeter on a flat surface, replace the cover and press the "Read" (right hand) button. Read the chlorine residual value on the digital screen and record the value in your field book.
- Also, record the sample identifier, the date and the time when the water sample was collected and when the chlorine residual was measured, and the initials of the person who did the reading.
- Note that the colourimeter records the most recent readings, plus the time when the readings were taken. These data can be accessed later to verify the field notes.
- When finished, dump the contents of the sample cell into a waste bottle and thoroughly rinse the sample cell with some of the water sample.
- Turn off the power on the meter.

All samples from the drinking water distribution system that give residual chlorine values less than 0.05 mg/L should be reported to the laboratory manager.

3.2 Turbidity

The **Hach 2100Q turbidity meter** is recommended for analysis in the field of the turbidity of water samples. Ensure that fully charged batteries are installed in the meter before taking it into the field. An instructional video for calibrating and operating this meter is available on YouTube: https://www.youtube.com/watch?v=zb_Ogaa8xDc

- Before going into the field, calibrate the instrument. Note that in the field, you can enter information using the settings button (wrench symbol) for the sampling the date, sample identifier code and the person that conducted the analysis of turbidity (optional).
- In the field, turn on the meter by pressing the power button. Press the up-arrow key to select the reading mode. For most water samples, you can use the "Normal" mode. However, if the sample is very turbid, you may have to scroll to the "Rapidly settling turbidity" mode.
- Gently invert the water sample collected in the field for analysis of water quality parameters.
- Fill the glass sample cell provided with the meter with water and then dump the water. Refill the sample cell, then cap.
- Wipe the outside of the sample cell with a paper towel to remove fingerprints and any traces of liquid. Gently invert the sample cell to resuspend any particles.
- Lift the cover on the turbidity meter and place the sample cell in the measuring chamber.
- With the meter on a flat surface, close the cover and press the right-hand button to take a reading of turbidity. Wait until the meter reading has stabilized, then record the turbidity value. Also record the sample identifier, the date and the time when the water sample was collected and the initials of the person who did the reading.
- Remove the sample cell and dump the contents. Turn off the power on the meter.

Note that this meter can store a large amount of information, including the sampling date, sample identifier, the operator ID and the results of the analysis. These data are recorded in the instrument during field collections and can be retrieved later in the lab to verify readings recorded in the field.

3.3 Salinity

The **HACH Pocket Pro+ Multi-2** instrument is recommended for analysis in the field of salinity. This is a multi-parameter meter that can also be used to measure the temperature, pH, conductivity and TDS in water samples. Although these parameters are not considered critical for water quality monitoring, it is useful to also collect these data.

Ensure that fully charged batteries are installed in the meter before taking it into the field. An instructional video for calibrating (pH, conductivity) and operating this meter is available on YouTube: https://www.youtube.com/watch?v=t-_Q0AqkQ34.

- Before the sampling trip, calibrate the instrument for pH and conductivity using solutions prepared from "Singlet" reagents supplied by Hach for calibration of pH and conductivity. These solutions should be prepared from deionized (or distilled) water.

- In the field, turn on the meter by pressing the power button.
- Pour some of the water sample into the small test container supplied with the instrument and dump.
- Refill the test container to the fill line and insert the probe into the water sample.
- Toggle between the water quality parameters using the arrow button at the top of the instrument until "Sal" appears at the top of the screen. Record the salinity reading and the water temperature. Also record the sample identifier, the date and the time when the water sample was collected and the initials of the person who did the reading.
- You can also toggle between the readings for pH, conductivity and TDS and record these values.
- Remove the probe from the sample and turn off the power on the meter.

3.3 Nitrate

It is recommended that initial screening of samples be done in the field using test strips that measure nitrate over a 0-50 mg/L range supplied by Hach (Product #2745425). These test strips give semi-quantitative information on whether nitrate and nitrite are present in a water sample. Make sure that the lid of the container holding the test strips remains tightly sealed when not in use as humidity can change the colour of the strips.

- Pour some of the water sample that was collected for nitrate analysis into the test container provided with the Pocket Pro+ Multi-2 meter and dump the water.
- Refill the test container with the water that was collected.
- Remove a test strip and holding it at one end, dip the other end slowly into the water sample. Cap the container holding the test strips immediately.
- Remove the test strip and continue to hold it between your fingers. Follow the instructions on the container for the length of time required for the colour to develop on the strip for analysis of both nitrite and nitrate.
- Compare the colour that develops on the strip to the colour comparator on the side of the test strip container to estimate the concentrations of both nitrite and nitrate.
- Record the approximate concentrations of nitrite and nitrate that you determined from the test strips. Also record the sample identifier, the date and the time when the water sample was collected and the initials of the person who did the reading.
- **For samples where the concentration of nitrate estimated from the test strips is greater than 3 mg/L NO_3-N, the samples should be re-analyzed in the lab to provide quantitative data on the nitrate concentration.**

Note that samples taken to the lab should be stored in a refrigerator for less than 48 hours before analysis. If analysis is delayed later than this, the samples must be preserved by acidifying to pH 2 using dilute sulfuric acid (5N) solution and returned to the refrigerator. Before analysis, the pH in the sample must be adjusted to 7 using dilute sodium hydroxide (5 N) solution.

The cadmium reduction method using test reagents supplied by Hach and analysis using the **HACH DR300 Pocket Colorimeter (Nitrate)** is recommended. The reagent powder sold by HACH in powder pillows (Product #LPV445) is for analysis over a high range of concentrations of nitrate (i.e., 0.4 to 30 mg/L). Results in the digital display are expressed as mg/L NO_3-N. This

method can be used for analysis of nitrate in drinking water, source waters and seawater. High concentrations of nitrite in the sample will interfere with this test. High nitrite may be present in groundwater that is hypoxic (i.e., low dissolved oxygen concentrations).

- The reagents in the powder pillows are hazardous to the environment (i.e., contain cadmium), so when the analysis is completed, dispose of the test sample in a waste container. Test solutions accumulated over time should be treated as hazardous waste.
- If water samples are stored in the lab, remove them from the refrigerator and let them reach room temperature. If water samples are analyzed in the field, they can be analyzed immediately.
- Turn on the meter with the power button and set it to Channel 1 by pushing the menu button, then the checkmark button, then the menu button again.
- From the water sample collected for analysis of nitrate, fill the sample cell with water and then dump the water. Add water again to the 10 mL line. Add the contents of one reagent pillow to the sample, cap it and shake vigorously to dissolve the reagent. Undissolved reagent will not affect the results.
- **Set and start a timer for a 5-minute reaction time.**
- While waiting for the reaction to be completed, prepare a blank sample by filling another sample cell with the water that was collected and dumping the water. Then refill the sample cell to the 10 mL line and cap.
- Wipe the outside of the blank sample cell with a paper towel to remove fingerprints and any traces of liquid.
- Remove the cover from the colourimeter and place the blank sample cell in the measuring chamber. Make sure the diamond-shaped marker is facing towards the digital screen.
- With the colourimeter on a flat surface, replace the cover and press the "0" button. The digital screen should read "0.00". Remove the blank sample cell and dump the contents.
- **Once the 5-minute reaction time is completed** for the water sample collected for analysis, wipe the outside of the sample cell with a paper towel, then place the sample cell in the measuring chamber (marker facing the screen) and replace the cover.
- With the colourimeter on a flat surface, press the button on the meter with the check mark symbol. Read the value on the digital screen and record the value in a notebook as mg/L NO_3-N. Also record the sample identifier, the date and the time when the water sample was tested, and the initials of the person who did the reading. These data should be immediately transcribed into the water quality data sheet (Appendix II), along with any other data collected in the field.
- Note that the colourimeter records the 10 most recent readings, plus the time when the readings were taken. These data can be accessed later to verify the data recorded manually.
- When finished, dump the contents of the sample cell into a waste bottle and thoroughly rinse with some of the water sample.
- When all samples have been analyzed, turn off the power on the meter.
- **All water samples that give nitrate values greater than 10 mg/L NO_3-N should be reported to the laboratory manager.**

3.4 M*icrobiological indicators*

The SOP described below is based on the recommendation to utilize the **IDEXX Colilert®** system for analysis of total coliforms and E. *coli* in water samples. The SOP described below applies to either the standard Colilert system that generates data after 24 hours of sample incubation, or the Colilert-18 system that generates data after 18 hours of incubation. An instructional video for using the Colilert system is available on YouTube:
https://www.youtube.com/watch?v=GlBVmOI4Taw.

- For water samples that are not expected to contain coliform bacteria (e.g., water from standpipes), the presence/absence test can be used for screening. For samples that are expected to contain coliform bacteria (e.g., surface water samples, raw drinking water), the quantitative test is recommended to estimate the numbers of total coliforms and E. *coli.*in the sample.
- Remember to check the expiry date of all reagents and the comparator.
- Wash and sanitize your hands before proceeding with the tests.
- Remove a Colilert snap pack of reagent from the strip.
- Remove the sample bottle collected in the field for microbiological analysis from the refrigerator and pour a subsample into the Colilert (100 mL) sample vial to the fill line. Be sure to not touch the rim of the Colilert sample vial with the lip of the sample bottle or with your fingers.
- Fold back the top of the reagent snap pack and pour the contents into the sample vial. Screw on the cap, being careful to not touch the inside of the cap, and gently shake the vial to dissolve the reagent.
- Label the cap on the sample vial with the site identifier.
- Return the sample bottle collected in the field to the refrigerator in case re-analysis is required (optimally within 24 hours).

Presence/absence test:

- Place the sample vials in an incubator set at 35ºC and incubate for 24 hours (or 18 hours for the Colilert-18 system). Record in the data sheet (Appendix III) the in-and-out time for the samples and the initials of the person that conducted the test.
- After incubation, compare the incubated sample against the colour comparator. If no yellow colour is observed, then the sample is negative for both total coliforms and E. *coli*. If this is the case, record the negative test result in the data sheet (Appendix III).
- If the sample has a yellow colour more intense than the comparator, then the sample is positive for total coliforms and should be examined for the presence of E. *coli*. Record the positive test result in the data sheet (Appendix III).
- If the sample has a slight yellow colour that is less intense than the comparator, the sample can be incubated for an additional 4 hours. If the sample is positive for coliforms, the colour will intensify.
- If the sample is positive for total coliforms, check the sample using UV light. Use protective eyewear if using a hand-held lamp or use a UV viewing cabinet. Place the sample vial 8-10 cm (3-4 inches) from the light source and look for blue fluorescence in a dark room. If fluorescence is greater than the comparator, then the sample is positive for E. *coli*. Record the negative or positive test result in the data sheet (Appendix III).

- If the sample is positive for E. *coli*, you may choose to use the sample stored in the refrigerator for a quantitative test (see below).
- **All samples that test positive for E. *coli* that were collected from the water distribution system (e.g., standpipes, domestic water faucets) should be immediately reported to the laboratory manager.**

Quantitative test:

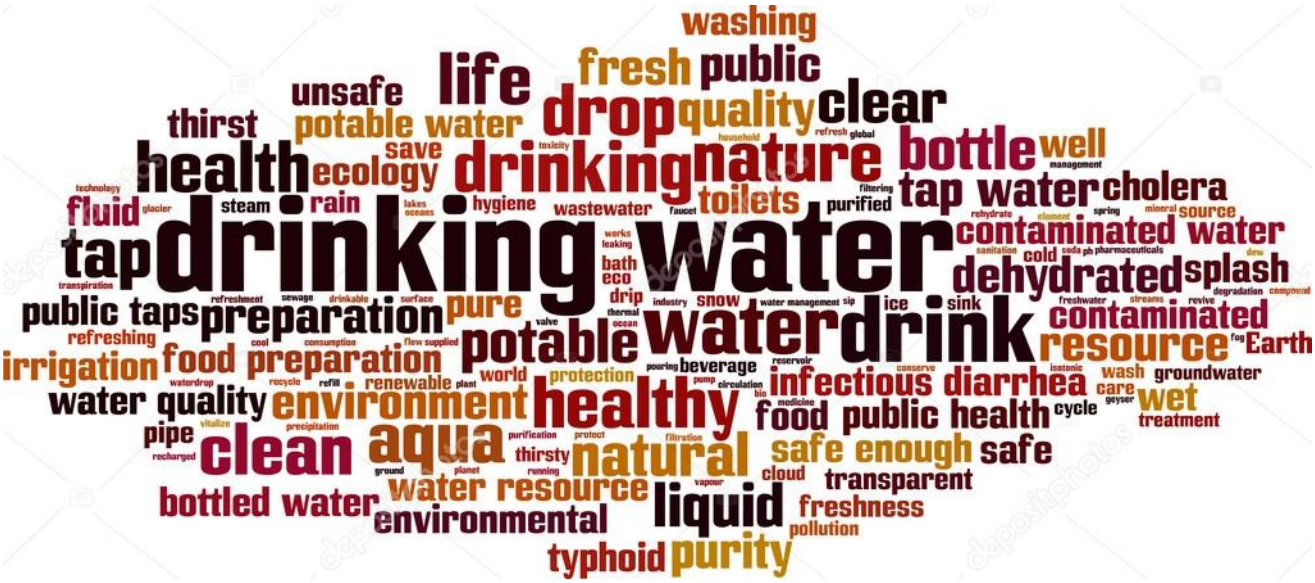
- Record information and prepare the test samples exactly as described above for the presence/absence test.
- Open the top of a Colilert Quanti-Tray and pour in the entire sample from the vial, being careful to not touch the opening of the Quanti-Tray with your fingers or with the lip of the sample vial.
- Place the Quanti-Tray in the rubber tray carrier with the sample cells facing down. Push the rubber tray carrier into the sealer. Retrieve the carrier from the other side of the sealer.
- After sealing, remove the Quanti-Tray from the rubber tray and label it with the sample identifier using a marker.
- Place all the Quanti-Trays in an incubator set at 35°C and incubate for 24 hours (or 18 hours for the Colilert-18 system). Record in the lab logbook the in-and-out time for the samples and the initials of the person that conducted the test.
- After incubation, compare the colour in the cells of the Quanti-Tray against the colour comparator. If none of the cells have a yellow colour, then the sample is negative for both total coliforms and E. *coli*.
- If a cell has a slight yellow colour that is less intense than the comparator, the Quanti-Tray can be incubated for an additional 4 hours. If the sample is positive for coliforms, the colour will intensify.
- Count the number of cells that have a yellow colour and record these data for total coliforms in the data sheet (Appendix III). The Quanti-Tray should then be examined for the presence of E. *coli*.
- Check the Quanti-Tray for the presence of E. *coli* using UV light. Use protective eyewear if using a hand-held lamp or use a UV viewing cabinet. Place the Quanti-Tray 8-10 cm (3-4 inches) from the light source and look for blue fluorescence in a dark room. If the fluorescence is greater than the comparator, then the sample is positive for E. *coli*.
- Count the number of cells that show blue fluorescence and record these data for E. *coli* in the data sheet (Appendix III).
- **All samples that tested positive for E. *coli* that were detected in samples collected from the water distribution system should be immediately reported to the laboratory manager.**
- Using the data recorded from your observations of positive cells in Quanti-Tray, determine the Most Probable Number (MPN) of total coliforms and E. *coli* in the sample using the MPN table below. Record the MPN values for each of the samples in the data sheet (Appendix III). These MPN data for 100 mL samples are directly comparable to the CFU/100 mL values generated using the membrane filtration method.

4. Recording data

The identifier code assigned to each sample must be used when recording all water quality data generated in the field and in the lab. As described in the SOPs, all data collected in the field must be recorded immediately in a field notebook. There is an option of also recording data in the field using a smart phone if data connectivity is available at all sampling sites.

In the laboratory, a logbook must be maintained to track when (date and time) the samples were delivered from the field to the lab and stored in the refrigerator, when they were removed from the refrigerator for analysis, and when they were analyzed. The person(s) handling the samples during each of these steps must also be recorded in the logbook. The date and times of calibration of all instruments should also be recorded in this logbook.

All water quality data must be transcribed from field books or from lab notebooks into a data sheet (Appendix II). All microbiological data for total coliforms and E. *coli* should be transcribed onto a separate data sheet (Appendix III). There is an option for lab personnel to directly transcribe the data using a lab computer.



Most Probable Number (MPN) table

Number of wells giving positive reaction	MPN/100 mL	Number of wells giving positive reaction	MPN/100 mL
1	1.0	26	36.4
2	2.0	27	38.4
3	3.1	28	40.6
4	4.2	29	42.9
5	5.3	30	45.3
6	6.4	31	47.8
7	7.5	32	50.4
8	8.7	33	53.1
9	9.9	34	56.0
10	11.1	35	59.1
11	12.4	36	62.4
12	13.7	37	65.9
13	15.0	38	69.7
14	16.4	39	73.8
15	17.8	40	78.2
16	19.2	41	83.1
17	20.7	42	88.5
18	22.2	43	94.5
19	23.8	44	101.3
20	25.4	45	109.1
21	27.1	46	118.4
22	28.8	47	129.8
23	30.6	48	144.5
24	32.4	49	165.2
25	34.4	50	200.5

If more than 50 cells are positive, report the MPN/100 mL as >200.5

MANUAL 2

Standard Operating Procedures for Collecting and Analyzing Recreational Waters for Intestinal *Enterococci*.

1. Introduction

The SOP described below is based on the recommendation to utilize the **IDEXX Enterolert®** system for analysis of intestinal *Enterococci* in recreational water samples. An instructional video for using the IDEXX Enterolert system for both presence/absence testing and for quantitative results is available on YouTube: https://youtu.be/oXyGL3SzXZw.

The WHO recommends analysis of intestinal *Enterococci*, rather than E. *coli,* because no statistical relationship has been established for the presence of E. *coli* that can support a guideline for recreational waters. In addition, the measurement of *Enteroccoci* in recreational waters is approved by the US EPA and is an official ASTM Method (i.e., Method #D6503-99).

The levels of intestinal *Enterococci* are the only water quality criteria for protecting the health of humans using recreational waters, except for monitoring of harmful algal blooms. All other water quality parameters (e.g., BOD, dissolved oxygen) are for the protection of aquatic life, and so, are not included in this SOP.

2. Preparing for sample collection

Clean and sterilize plastic sample bottles and add labels. Confirm the four-digit sample identifier number to be applied to all samples during the sampling trip. Assemble a field kit that includes:

- Coolers with cold packs to store the samples in the field.
- An appropriate number of plastic sterile sample bottles (250 or 500 mL) with labels attached. Sterilization procedure listed below.
- Waterproof field notebook, pens and labelling markers.
- Sanitary wipes or hand sanitizer.
- Smart phone for recording field data (optional).

Sterilized bottles: *Pre-sterilized sample bottles are available from commercial suppliers. However, if sample bottles are to be re-used, clean them thoroughly with laboratory grade soap and water, then rinse the bottles with deionized (or distilled) water and air dry. Loosely fit the cap on each bottle (do not tighten) and sterilize in an autoclave at 120^{o}C with 15 psi pressure for 20 minutes. Remove the sample bottles from the autoclave, allow to cool, tighten the cap on each bottle and apply a label.*

3. Sample Collection

Water samples are collected by wading into knee-deep water and collecting a water sample from below the water surface by hand or by using an extendable sample pole. See the safety precautions for wading (below).

- Sanitize your hands.
- Wade knee-deep into the water at a point on a recreational beach which is typically frequented by bathers. Be careful when wading to not disturb and resuspend bottom sediments.
- Collections by hand: Uncap the sample container and submerge the bottle neck-first into the water. The container should be submerged to 0.3 m (about elbow depth). Avoid touching rocks or other solid objects with the sample container. Face the opening of the container toward the prevailing beach current and allow it to fill with water. If there is no current, create a current artificially by pushing the bottle forward horizontally in the direction away from the hand.
- Collections with a sampling pole: Two people can collect a sample using an extendable sampling pole, as described previously for the collection of surface water samples.
- Collect the sample bottle and pour off some of the sample to allow for ample air space in the container to facilitate mixing by shaking. Replace the cap immediately.
- Place the sample in a cooler for transport back to laboratory.
- Record location, sample identifier, time and date in your field notebook or record the information on a Smart phone.

4. Safety Precautions - Wading

Wading should only be done when beach conditions are safe. Please follow local beach warning flags and signs. **Always wear a life jacket when wading** and **do not wade alone**. Never wade in water that is deeper than 3 feet under any circumstances. Wading should **NEVER** be done in unsafe weather conditions such as when there are high waves, or lightning and thunder.

5. Analyzing water samples

- For water samples that are not expected to contain intestinal *Enterococci*, the presence/absence test can be used for screening. For samples that are expected to contain intestinal *Enterococci* (e.g., sampling near a storm water drain or near wastewater discharges), the quantitative test is recommended to estimate the numbers of *Enterococci* in the sample.
- Remember to check the expiry date of all reagents.
- Wash and sanitize your hands before proceeding with the tests.
- Remove an Enterolert snap pack of reagent from the strip.
- Remove the sample bottle collected in the field for microbiological analysis from the refrigerator **NOTE - Marine water samples must be diluted at least tenfold to reduce the salinity of the sample. Pour 20 mL of the sample into a pre-sterilized 200 mL graduated cylinder, then make up to 200 mL with deionized (or distilled) water. Always prepare a blank using the dilution water to check if there is bacterial contamination.**
- Pour a subsample into a sterile Enterolert sample vessel and fill to the 100 mL fill line. Be sure to not touch the rim of the vessel with the lip of the sample bottle or with your fingers.
- Fold back the top of the reagent snap pack and pour the contents into the sample vial. Screw on the cap, being careful to not touch the inside of the cap, and gently shake the vial to dissolve the reagent.

- Label the cap on the sample vial with the site identifier.
- Return the sample bottle collected in the field to the refrigerator in case re-analysis is required (optimally within 24 hours). This may be the case if there is a positive in the presence absence test. Remaining water samples could be used for quantitative testing.

Presence/absence test:

- Place the sample vials in an incubator set at 41 ± 0.5°C and incubate for 24 hours. Record in the data sheet (Appendix IV) the in-and-out time for the samples and the initials of the person that conducted the test. Note that this incubation temperature is higher than the temperature of incubation for analysis of total coliforms and E. *coli.*
- After incubation, check the sample for the presence of *Enterococci* using UV light. Use protective eyewear if using a hand-held lamp or use a UV viewing cabinet. Place the sample vial within 13 cm (5 inches) from the light source and look for blue fluorescence in a dark room. If there is any blue fluorescence, the sample is positive for *Enterococci.* Lack of fluorescence indicates a negative result. Record the negative or positive test result in the data sheet (Appendix IV).
- If the sample is positive for *Enterococci,* you may choose to use the sample stored in the refrigerator for a quantitative test (see below),
- **All samples that test positive for *Enterococci* should be immediately reported to the laboratory manager to decide if a quantitative analysis is required.**

Quantitative test:

- Record information and prepare the test samples exactly as described above for the presence/absence test.
- Open the top of a Enterolert Quanti-Tray and pour in the entire 100 mL sample from the vial, being careful to not touch the opening of the Quanti-Tray with your fingers or with the lip of the sample vial.
- Place the Quanti-Tray in the rubber tray carrier with the sample cells facing down. Push the rubber tray carrier into the sealer. Retrieve the carrier from the other side of the sealer.
- After sealing, remove the Quanti-Tray from the rubber tray and label it with the sample identifier using a marker.
- Place all the Quanti-Trays in an incubator set at 41 ± 0.5°C and incubate for 24 hours. Record in the lab logbook the in-and-out time for the samples and the initials of the person that conducted the test.
- Check the Quanti-Tray for the presence of *Enterococci* using UV light. Use protective eyewear if using a hand-held lamp or use a UV viewing cabinet. Place the Quanti-Tray 13 cm (5 inches) from the light source and look for blue fluorescence in a dark room.
- Count the number of cells that show blue fluorescence and record these data in the data sheet (Appendix IV).
- Using the data recorded from your observations of positive cells in Quanti-Tray, determine the Most Probable Number (MPN) of *Enterococci* in the sample using the MPN table (see previous). Record the MPN values for each of the samples in the data sheet (Appendix IV). **NOTE:** Multiply the MPN value by the dilution factor to obtain the proper quantitative result for marine samples
- **All samples that tested positive for *Enterococci* and generated quantitative results of >200 MPN should be immediately reported to the laboratory manager.**

Quality control

It is recommended that a positive control test be used with each lot of IDEXX-Enterolert samples. In this case, IDEXX-QC *Enterococci* (IDEXX Catalog #UN3373-WQC-ENT) solution can be used following the presence absence or Quanti-Tray enumeration procedure described above.

6. Recording data

The identifier code assigned to each sample must be used when recording all water quality data generated in the field and in the lab. As described in the SOPs, all data collected in the field must be recorded immediately in a field notebook. There is an option of also recording data in the field using a smart phone if data connectivity is available at all sampling sites.

In the laboratory, a logbook must be maintained to track when (date and time) the samples were delivered from the field to the lab and stored in the refrigerator, when they were removed from the refrigerator for analysis, and when they were analyzed. The person(s) handling the samples during each of these steps must also be recorded in the logbook. All microbiological data should be transcribed onto a separate data sheet (Appendix IV). There is an option for lab personnel to directly transcribe the data using a lab computer.

ADDITIONAL RESOURCE DOCUMENTS

Guidelines on recreational water quality. Volume 1: (2021) Coastal and fresh waters. World Health Organization, Geneva, Switzerland; License: CC BY-NC-SA 3.0 IGO.

IDEXX Water Control Laboratory (2015) Enterolert Procedure. IDEXX Laboratories, Inc., Westbrook, Maine, USA

State of Hawaii Department of Health, Environmental Management Division, Clean Water Branch, Monitoring and Analysis Section (2012) Beach Sampling Protocol. Available on-line.

MANUAL 3

Standard Operating Procedures for Collecting and Analyzing Samples of Treated Municipal Wastewater

1. Preparing for Collecting Samples

Prior to collection and analysis of wastewater samples, calibrate all instrumentation according to the manufacturers' instructions. Label clean sample bottles with the appropriate sample identifiers. The wastewater field kit should include the following items:

- Cooler with cold packs or ice.
- Cleaned and sterilized plastic sample bottles (1000 mL).
- Extension sampling pole.
- Bleach solution (15 mL of household bleach added to 100 mL tap water) and a roll of paper towels.
- Powder free latex gloves.
- N95 masks.
- Safety glasses and/or face shields.
- Sanitary wipes and/or hand sanitizer
- Container for solid wastes.
- Waterproof field notebook, pens and labelling markers
- Smart phone for recording field data (optional)

Cleaning and sterilizing the sample bottles

Thoroughly clean sample bottles and lids with phosphate free laboratory grade soap and warm water and then rinse with deionized (or distilled) water. Air dry and then cap loosely with the lids. Sterilize the bottles in an autoclave at 120°C with 15 psi pressure for at least 20 minutes. After sample bottles are cooled, tighten the lids and affix labels on the bottles.

Health and safety precautions

Personnel involved in collecting samples of wastewater must be vaccinated against a range of waterborne diseases, including hepatitis A and B, typhoid and cholera. **Before entering the plant**, personnel must put on latex gloves, N95 masks, safety glasses and/or face shields. Many wastewater treatment plants also require visiting personnel to wear a certified hardhat and safety boots. While in the plant, personnel should avoid touching surfaces (e.g., railings) to minimize the risk of contamination. **After leaving the plant**, personnel should safely remove their gloves and masks for disposal, and disinfect their hands, safety glasses and/or face shields and hardhats. All solid wastes should be disposed of in a solid waste container.

2. Collecting Wastewater Samples

- The samples will be collected from the stream of **final treated effluent** in the wastewater

treatment plant. If the treated wastewater is disinfected prior to discharge, collect the sample from the wastewater stream just prior to the chlorination stage.

- Effluent samples can be collected as "grab" samples or as composite samples. Grab samples only represent the composition of the wastewater at the time of collection. The flow and composition of wastewater is changing constantly. For instance, flows are typically lowest at night (and the sewage more concentrated), while flows increase in the morning (and the sewage is more dilute). Composite samples are time-integrated samples made up of sub-samples of equal volume taken at specific intervals over a standard period of time, which is usually 24-hours. If the wastewater treatment plant is equipped with a composite sampler for collection of final treated effluent, this is the preferred option. **If samples are to be analyzed for cBOD5 and/or COD, the sample holding compartment of the composite sampler should be refrigerated.**
- Sample bottles must be clearly labelled with a 4-digit sample identifier and a 3-letter code for the wastewater treatment plant site. The time and date must be recorded along with all relevant details of the location and the sampling conditions. Heavy rains will dilute the wastewater in clarifiers and aeration ponds that are open to elements, so avoid sampling during periods of heavy rain.
- If there is a composite sampler available to collect a sample of final treated effluent, personnel from the plant should begin 24-hours prior to the scheduled sample pickup to collect the sample in the composite sampler. At sample pickup, work with plant personnel to transfer subsamples from the composite sampler to a labelled sample bottle.
- Grab samples of final treated wastewater can be collected from the effluent stream by two people using an extendable sampling rod. For the procedure, see the SOP for collecting a surface water sample using the sampling rod, except wear disposable latex gloves when collecting the sample. During wastewater collection, care should be taken to avoid disturbing materials adhering to the surfaces of pipes, chambers and channels.
- Securely seal the sample bottle with the lid following sampling and then disinfect the outside with the bleach solution. Place the bottle in a cooler with ice packs for transport to the lab. Dispose of paper towels soaked in bleach in a solid waste container.
- In the lab, store all sample bottles in a refrigerator until they are removed for analysis of wastewater parameters. Keep the storage area for wastewater samples separate from other samples (e.g., drinking water) to avoid cross-contamination. As a general rule, water samples should be analysed within 24-hours of sample collection.

3. Analysing Wastewater Samples

The wastewater quality parameters recommended for monitoring include:

- Total suspended solids (TSS)
- Carbonaceous biochemical oxygen demand (cBOD5) **OR**
- Chemical oxygen demand (COD)

SOPs for these parameters are provided below. TSS should be analyzed for all samples, but either cBOD5 of COD can be analysed, depending on availability of equipment, training requirements, time constraints, etc.

Health and safety precautions

Personnel involved in analysing samples of wastewater must be vaccinated against a range of waterborne diseases, including hepatitis A and B, typhoid and cholera. **Before handling wastewater samples**, lab personnel must put on latex gloves, N95 masks, safety glasses and/or face shields. All procedures for preparing samples for analysis should be done in a functioning fume hood. **After handling wastewater samples**, lab personnel should safely remove their gloves and masks for disposal and disinfect safety glasses and/or face shields. All surfaces that were touched during sample preparation (e.g., on/off switches, vacuum pumps, etc.) should also be disinfected. All solid wastes should be disposed of in a container for biological waste.

A) Total Suspended Solids (TSS)

The SOP below describes the procedures for determining the amounts of suspended solids in samples of treated municipal wastewater using a weight differential calculation.

An instructional video for analysis of TSS in liquid samples is available on YouTube:
https://www.youtube.com/watch?v=_fKGM040wvI
However, please note that the liquid sample in this video is not a microbiological hazard and so no health and safety precautions were taken in the video. The amount of solids in the sample is also high so only a small volume (i.e., 5 mL) was collected for TSS analysis.

Equipment and supplies

All equipment and supplies listed below are recommendations only. Equivalent components are also acceptable. Note that the volume of the wastewater sample that can be filtered depends on the size of the filter (and the filtration apparatus) and the amounts of suspended solids in the sample. If a wastewater treatment plant is operating properly, the TSS values in final treated effluent should be less than 35 mg/L, but under some circumstances, samples may contain higher amounts of suspended solids.

- Whatman GF/C microfibre filters, 1.2 µm pore size (47 mm or 70 mm diameter)
- Nalgene or glass filtration apparatus (filter holder and sample reservoir); Size depends on the diameter of the filter.
- Side-arm Nalgene Erlenmeyer flask (5000 mL) with rubber stopper and centre hole
- Graduated cylinder (500 mL)
- Aluminum weighing dishes
- Drying oven
- Dessicator with anhydrous calcium sulphate dessicant
- Analytical balance capable of accurate weighing to 0.001 grams*
- Vacuum pump
- Tweezers
- Wash bottle with deionized (or distilled) water
  The Optima Milligram precision balance (110 g x 0.001 g) is recommended. It must be placed on a vibration free bench in a draft free location.

Procedure

1. Before filtering the samples for analysis of TSS, decide on the number of filters you will need to analyse all wastewater samples plus a blank. Also, set aside the appropriate number of aluminum weighing dishes.
2. Transfer one filter into each aluminum weighing dish and place the dishes in a drying oven set at a temperature of 103-105°C. Dry the filters for at least one hour. The filters can also be left to dry overnight.
3. Using tweezers, transfer the dishes containing the dried filter discs to a dessicator and allow to cool. Wait at least 30 minutes before removing the dishes and filter papers from the dessicator. Label the side of each aluminum dish with an identifying number.
4. Zero the analytical balance and using tweezers, transfer the filter from the dish to the analytical balance. Place the filter in the center of the weighing platform. Once the weight stabilizes, record the weight of the filter paper and the identifying number on the dish in the suspended solids data sheet (Appendix 4). Replace the filter back into its corresponding aluminum dish.
3. Repeat step 3 with all filters corresponding to the number of samples to be analysed plus a blank. If there is a delay in weighing all of the filters, place them back in the dessicator until they can all be weighed.

AT THIS POINT, ALL WORK MUST BE DONE IN A FUME HOOD, FOLLOWING HEALTH AND SAFETY PROTOCOLS.

4. Place the filter holder of the filtration apparatus on top of the side-arm Erlenmeyer flask. Hook up the vacuum hose to the side arm of the flask.
5. Once all the filters have been accurately weighed, begin filtering samples. Place the first weighed filter on top of the filter holder, and then attached the sample reservoir.
6. Turn on the vacuum pump and pour 500 mL of deionized (or distilled) water into the sample reservoir. This is your *blank* sample. Filter, then turn off the pump and remove the reservoir. With tweezers, remove the blank filter, and place it back into a numbered aluminum dish.*
7. Mix a wastewater sample well by shaking the sample bottle, then pour 500 mL into the graduated cylinder. Large floating particles or nonhomogeneous materials that sink to the bottom should be excluded from the sample if it is determined that their inclusion is not representative.
8. Turn on the vacuum pump and pour the sample into the reservoir in approximately 100 mL aliquots. The volumes filtered can be adjusted if the filter clogs. If less than 500 mL of the sample is filtered, take note of the volume still left in the graduated cylinder and calculate what volume passed through the filter before it clogged. Record this in the suspended solids data sheet (Appendix V).
9. Using the wash bottle, wash the inside of the reservoir with deionized (or distilled) water and allow to pass through the filter. Turn off the pump, remove the reservoir and with tweezers, remove the filter containing the filtered solids, and place it back into the numbered aluminum dish.*
10. Repeat steps 7-9 for each wastewater sample. **NOTE: Do not let the Erlenmeyer flask get more than 2/3 full of wastewater (i.e., about 300 mL) before emptying it.** This will avoid wastewater being aspirated into the vacuum pump. Dispose of the filtered

wastewater collected in the flask in a liquid waste container.

11. After all samples have been filtered, turn off the vacuum pump.

12. In the suspended solids data sheet (Appendix V), record the sample identification number next to the number assigned to the aluminum dish. After all samples have been filtered, place the dishes in the oven at 103-105°C for drying for at least 1 hour.

AT THIS POINT, ALL REMAINING WORK CAN BE DONE ON THE LAB BENCH, BUT FOLLOWING HEALTH AND SAFETY PROTOCOLS.

13. After use, the filtration apparatus and Erlenmeyer flask should be thoroughly cleaned by personnel wearing rubber gloves using soap and water and then rinsed with deionized (or distilled) water and air dried. Sterilize the wastewater container in the autoclave before flushing the contents down the drain.

14. After one hour, remove all samples from the oven and place them in a dessicator to cool to room temperature (30 minutes).

15. Weigh all the filters containing the dried suspended solids. Record all of the weights and the identity of the samples in the suspended solids data sheet (Appendix V). Calculate the TSS value in mg/L (see below) and enter that value on the data sheet. **All treated wastewater samples with suspended solid values greater than 50 mg/L should be reported to the laboratory manager.**

**If during handling, the filter is ripped or a piece is removed, then it must be discarded and a fresh sample filtered.*

Calculations

1. Determine the weight of the solids on the filter in grams (g):
Weight of filter with dried solids in grams - Weight of filter paper in grams

2. Convert grams to milligrams (mg):
Weight of solids in grams x 1000 = Weight of solids in milligrams

3. Convert the weight to represent a 1 litre (1000 mL) sample in mg/L:

$$\text{Weight of solids in milligrams} \times \frac{1000\ \text{mL}}{\text{sample volume (mL)}}$$

4. *Example:*
Weight of filter = 0.150 grams
Weight of filter and dried solids = 0.160 grams
Volume of sample filtered = 500 mL
0.160 g - 0.150 g = 0.010 g = 10 mg

$$10\ \text{mg} \times \frac{1000\ \text{mL}}{500\ \text{mL}} = 20\ \text{mg/L}$$

B) Biochemical Oxygen Demand

Biochemical oxygen demand includes both carbonaceous oxygen demand (cBOD) and nitrogenous oxygen demand (nBOD). The cBOD value more closely represents oxygen demand associated with microbial degradation of the organic constituents in wastewater and therefore is widely used to assess the quality of treated wastewater prior to discharge. Therefore, the SOP below describes the procedures for determining the carbonaceous biochemical oxygen demand within municipal wastewater over a 5-day incubation period (i.e., cBOD5).

An instructional video for measuring BOD5 is available on YouTube:
https://www.youtube.com/watch?v=ECUoozfg8BM
Note that the video includes instructions for "seeding" samples to ensure that there is sufficient reduction in BOD over the 5 days of incubation, but this is not considered necessary for the instructions below. Note that it is assumed that all treated municipal wastewater samples will not require pH adjustment (i.e., they are between pH 6 and 8). Nitrification inhibitor is added to the samples in order to measure only cBOD5.

Equipment and supplies

All equipment listed below are recommendations only. Equivalent components are also acceptable.

- Glass incubation bottles with glass stoppers (300 mL) and plastic caps. Before use, clean all bottles with phosphate-free lab soap, rinse thoroughly with deionized (or distilled) water, and air dry.
- Incubator set at a temperature of $20^{\circ}C \pm 1^{\circ}C$. Exclude all light to prevent production of oxygen by photosynthetic microorganisms.
- Bench top dissolved oxygen meter with probe*
- HACH nutrient pillows (for 6 L); Product #1486266
- HACH nitrification inhibitor with a dispenser cap, Product #253335
- Deionized (or distilled) water
- Large plastic carboy (20 L)
- Graduated cylinders (100 mL and 200 mL)
- Volumetric flask (1000 mL)
- Aeration pump and air stones.

***** A suitable meter is the HACH HQ440D benchtop meter with LBOD101 probe

Sample storage

Ideally, BOD analysis should be initiated within 6 hours of collection. If circumstances prevent testing within this period, samples should be stored in a refrigerator. In no instance should testing be carried out on samples that have been stored in the refrigerator for more than 24 hours. Record any sampling information contained on the sample label on the lab data sheet (Appendix VII). If testing is carried out within 2 hours of collection, cold storage is not necessary.

Procedure

Preparation of dilution water

1. Place 6 litres of deionized (or distilled) water into a 20 L carboy.
2. Add the contents of one nutrient pillow to the water and shake to mix the nutrients.
3. Aerate the dilution water by bubbling air through the water by means of a pump connected to an air stone with a length of flexible tubing. The water can also be aerated by vigorous agitation. Aeration of the dilution water must continue until the dissolved oxygen content is at least 8.0 mg/L. The water temperature should be 20˚C ± 1˚C before making dilutions.

Sample dilution and analysis

- Wastewater samples should be diluted to a concentration that will not reduce the DO reading to below 1 mg/L but will deplete DO by at least 2 mg/L after 5 days of incubation. Tests that do not meet these criteria need to be repeated.
- For a sample of deionized dilution water prepared as a "Blank", oxygen depletion over the 5 day incubation period should not exceed 0.2 mg/L. If oxygen depletion in the blank is greater than this amount, a more highly purified water source should be used or bottle cleaning practices should be improved. If the blank analysis is unreliable, the entire sample run must be rejected.

THE SAMPLE DILUTION STEP AND PREPARATION OF BOD BOTTLES SHOULD BE CONDUCTED IN A FUME HOOD USING HEALTH AND SAFETY PROTOCOLS

1. The suggested dilution for final treated wastewater samples is a 20% (volume to volume) dilution. Transfer 200 mL of each wastewater sample into a 1000 mL volumetric flask and top up to the mark in the flask with deionized (or distilled) water. However, if the test results using this dilution do not meet the criteria described above, then the sample may require more dilution (i.e., if DO is depleted to below 1 mg/L) or less dilution (i.e., if DO depletion is less than 2 mg/L).
2. Prepare a *blank* sample by filling a 300 mL BOD bottle with dilution water only. Add two "shots" of nitrification inhibitor (0.08 g in each shot) to the bottle. Fill the bottle so that the bottom of the meniscus lines up with the bottom of the bottle neck. Label it as the blank.
3. For the sample BOD bottles, label them with a number (Appendix VII). Carefully add 60 mL of a diluted wastewater sample to a 300 mL bottle. Add two "shots" of nitrification inhibitor to the bottle. **Note:** Add the aerated dilution water slowly to the bottle to prevent the introduction of air bubbles.
4. Record all sample information in the BOD data sheet. Be sure to include information on the dilution used and any other pertinent information (Appendix VII).
5. When all sample bottles have been filled, gently tap the side of the BOD bottles to dislodge any trapped air bubbles.

AT THIS POINT, THE PROCEDURES CAN BE MOVED TO THE LAB BENCH.

6. Calibrate the DO meter and probe according to the manufacturer's instructions. Insert the probe slowly into the *blank* BOD bottle. Turn the mixer switch on the probe to the ON position. Allow the reading to stabilize (i.e., when the screen stops flashing the DO value) and record the result.

This usually takes about 5 minutes. Record the blank result on the data sheet (Appendix VII). Place the stopper into the top of the blank bottle. There should be sufficient sample in the bottle to push up over the stopper. This water will keep it airtight during the 5-day incubation period. Add the plastic cap.

7. Rinse the probe with deionized (or distilled) water and take a DO reading for each of the sample bottles, as described above. After taking the initial DO reading, place the glass stopper into the top of each bottle. There should be sufficient sample in the bottle to push up over the stopper. Add the plastic cap. Rinse the DO probe with deionized (or distilled) water between each sample reading. Enter all data into a data sheet (Appendix VII)
8. Place the blank and the sample bottles in the incubator at 20˚C for 5 days. Check the incubator temperature every day.
9. After 5 days, take the BOD bottles out of the incubator. Remove the plastic caps and drain off the water seal from the top flange into a sink.
10. Read the DO values in the bottles using the calibrated DO meter (as described above) and record the readings in the BOD data sheet (Appendix VII)
11. Calculate the cBOD5 values using either a manual calculation or a calculation programmed into an Excel spreadsheet and enter into a data sheet (Appendix VII).
12. **All samples that generate cBOD5 values greater than 25 mg/L should be reported to the laboratory manager.**

cBOD5 calculation

The value can be manually calculated using the following equation:

$$\text{cBOD5 (mg/L)} = \frac{(D_0 - D_1) - (B_0 - B_1)}{P}$$

D_0 = Initial dissolved oxygen reading in sample
D_1 = Final dissolved oxygen reading in sample
B_0 = Initial dissolved oxygen reading in blank
B_1 = Final dissolved oxygen reading in blank
P = Percent concentration of sample as a decimal (e.g., 20% = 0.20)

Computer generated calculation – The BOD can be calculated via an Excel programme worksheet.

C) Chemical Oxygen Demand (COD)

The SOP below describes the procedures for determining the chemical oxygen demand in municipal wastewater samples using the HACH DR3900 spectrophotometer and a HACH digestor and reagents. Equivalent instruments are also acceptable. Ensure that the spectrophotometer is calibrated based on the manufacturer's instructions prior to usage.
An instructional video for determining COD is available on YouTube:
https://www.youtube.com/watch?v=ImtXAHZpkFo.

Note that the reagents in the digestion vials used for this test are corrosive and a chemical hazard (contain mercury). Therefore, care must be taken to avoid contact with the reagents and to dispose of the reagents as hazardous waste. Disposable latex gloves, safety glasses, a non-flammable lab coat and closed-toed shoes must be worn by lab personnel. A safety shield should be set up in front of the COD reactor when in use to protect lab personnel.

Equipment and supplies

All equipment listed below are recommendations only. Equivalent components are also acceptable.

- HACH COD digestion vials (20 – 1500 ppm) containing reagent. Product #2125915-CA.
- HACH DRB200 single block COD reactor
- Safety shield
- HACH DR3900 spectrophotometer
- HACH COD vial adapter for spectrophotometer. Product #4846400
- 0-1 mL adjustable volumetric pipette and disposable pipette tips
- Test tube rack
- Graduated cylinder (100 mL)
- Deionized (or distilled) water
- Lab timer
- Vortex mixer (optional)
- Household blender (if required).
- pH meter and concentrated sulfuric acid (if required for sample preservation)

Sample storage

Plastic sample bottles that are to be used for COD analysis must be scrupulously cleaned prior to sampling to remove any residues that could contribute to chemical oxygen demand. If the refrigerated samples cannot be analysed within 24 hours of collection, they must be acidified to a pH of less than 2 (using 1 N sulfuric acid) and stored in the refrigerator. Samples stored under these conditions can be maintained for up to 28 days. If significant volumes of acid are used to lower the pH, the final COD reading should be adjusted to compensate for the dilution.

Procedure

Digestion

1. Turn on the COD reactor and preheat to 150°C.
2. For samples containing large amounts of suspended solids, homogenize 100 mL of sample in a blender for 30 seconds. **This should be done in a fume hood using safety protocols for handling wastewater samples.**
3. Remove the cap from a COD vial. Pipette 0.5 mL of deionized (or distilled) water into the vial as a blank. **Note:** The blank prepared for the COD assay is stable for up to one week. Label the cap of the vial to indicate that it is a blank.
4. **In a fume hood**, pipette 0.5 mL of each wastewater sample into individual COD vials.*

5. Replace the vial caps tightly and label the caps of the vials with the appropriate sample identifier. Wipe the outside of the vials with a paper towel to remove any moisture.
6. Hold the vials over the sink and invert several times to mix the sample with the reagents. A vortex mixer may also be used, if available.
7. Place the vials in the reactor chamber. Be sure the temperature has reached 150°C before placing the vials in the reactor. Position the safety shield in front of the reactor. Set the timer to 60 minutes.
8. When the samples have digested for 1 hour, remove the vials and place them in a test tube rack for two minutes to air cool. Then cool the vials to room temperature in a cool water bath or under running tap water. This usually takes about 3 minutes.
9. Remove the vials from the water and wipe with a clean paper towel. Invert the vials several times to mix or use a vortex mixer.

   **Samples with COD values above the upper limit of the vial being used (e.g. sample COD>1500 mg/L) must be diluted with deionized water and homogenized in order to fall close to the midpoint of the detectable range. However, if a wastewater treatment plant is operating properly, the COD values for treated wastewater should be less than 125 mg/L.*

Colourimetric measurement

10. Insert the COD vial adapter into the sample cell module by sliding it under the thumbscrew and into the alignment grooves of the DR3900 spectrophotometer. Fasten with the thumbscrew.
11. From the spectrophotometer main menu, select the key under the HACH Program icon. At the prompt, type in the Method 432, then press "Enter".
12. Clean the outside of the blank vial with a paper towel and place into the sample cell of the spectrophotometer. Press the "Zero" key.
13. Remove the blank sample tube. Clean the outside of a sample vial with a lint-free towel and place into the sample cell of the spectrophotometer.
14. Press the "Read" key. The display will show the COD value in mg/L.
15. Tabulate the COD results in the COD data sheet (Appendix VI).
16. If the word "OVER" appears after the reading, the COD value in the sample is too high and a dilution is required. Dilute with deionized/distilled water and repeat immediately.
17. If the sample was diluted prior to reading, multiply the final reading by the inverse of the dilution. For example, if 5 mL of original sample was diluted with deionized water to a volume of 25 mL, then the spectrophotometer reading must be multiplied by a value of 5.
18. **If the COD value is greater than 125 mg/L, inform the laboratory manager.**

ADDITIONAL RESOURCE DOCUMENTS

Standard Methods for the Examination of Water and Wastewater, 23th edition, (2017) Rice et al, American Water Works Association, Washington, D.C., USA.

Hach Water Analysis Handbook, 5th edition (2017) Hach Company, Loveland, CO, USA. Available on-line at: https://www.hach.com/wah

MANUAL 4

Standard Operating Procedures for Collecting and Analyzing Samples of Drinking Water After Disinfection for Total and Residual Chlorine

1. Introduction

The WHO recommends that drinking water disinfected with chlorine have a minimum level of total chlorine of 2.5 mg/L and a minimum level of residual (free) chlorine of 0.5 mg/L prior to entering the water distribution system. This is recommended to maintain residual chlorine levels of at least 0.2 mg/L at the furthest points in the water distribution system. However, note that some jurisdictions allow residual chlorine levels to be as low as 0.05 mg/L in the water distribution system. The temperature, turbidity and the pH of the water have a significant effect on the efficiency of chlorine as a disinfectant. For optimal disinfection, the turbidity of the raw water before disinfection should be <5 NTU and the pH level between 6.8 and 7.2. The data for these parameters should be available from the Environmental Health Lab. Monitoring at least on a daily basis for total and residual chlorine is recommended.

2. Collecting Samples

- The SOP below is for analyzing samples of drinking water that have total or residual (free) chlorine values between 0.1 to 8.0 mg/L, which is considered the "high range".
- The procedures are described for analysis using the **Hach DR300 Pocket Colorimeter (Chlorine),** although other equivalent instruments can be used.
- Ideally, collect samples in clean glass bottles. Plastic containers may have a large chlorine demand.
- It is optimal to rinse the sample bottles with deionized or distilled water after use. If this is not possible, rinse the bottles with raw water collected prior to disinfection.
- Make sure to get a representative sample. If the sample is taken from a spigot or a faucet, let the water flow for at least 5 minutes. Let the sample bottle overflow with the water and then put the cap on the sample bottle so that there is no headspace (air) above the sample.
- Analyze the samples immediately. The samples cannot be preserved for later analysis.

3. Sample analysis

The SOP described below is based on use of the Hach DR300 Pocket Colorimeter (Chlorine) for testing of total and residual (free) chlorine in the field. Ensure that fully charged batteries are installed in the meter before use. The method requires addition of DPD free chlorine reagent (Product # 2105569-CA) or DPD total chlorine reagent (Product # 2105669-CA) to the water sample prior to analysis.

- Turn on the meter with the power button. To set the instrument at the high range, press the "up" button: ▲
- From the water sample collected after chlorine disinfection, fill the sample cell with water and then dump the water. Refill the sample cell to the 10 mL line, then cap. This is the "blank" sample.
- Wipe the outside of the blank sample cell with a paper towel to remove fingerprints and any traces of liquid.
- Remove the cover from the colourimeter and place the blank sample cell in the measuring chamber. Make sure the diamond-shaped marker is facing towards the digital screen. Place the cap over the sample.
- With the colourimeter on a flat surface, press the "0" button. The digital screen should read "0.00".
- Remove the blank sample cell and dump the contents.
- From the water sample collected after disinfection for analysis, fill two sample cells with water then dump. Refill both sample cells to the 10 mL line. One sample will be for analysis of <u>total chlorine</u> and the other sample will be for the analysis of <u>residual (free) chlorine</u>.
- To one sample, add the contents from <u>two packets</u> of DPD <u>total chlorine</u> reagent. Cap the sample cell and shake gently. Note that the accuracy of the reading is not affected by undissolved reagent. Start a timer set at 3 minutes.
- To the other sample, add the contents of <u>two packets</u> of DPD <u>free chlorine</u> reagent. Wipe the outside of the sample cell with a paper towel, then place the sample cell in the measuring chamber and replace the cap. **This needs to be done within <u>one minute</u> of adding the DPD reagent to the sample.**
- With the colourimeter on a flat surface, press the button on the meter with the check mark symbol. Read the chlorine residual value (mg/L) on the digital screen and record the value in your field notebook. When finished, dump the contents of the sample cell into a waste bottle and thoroughly rinse the sample cell with deionized or distilled water.
- For the remaining sample, wipe the outside of the sample cell with a paper towel, then place the sample cell in the measuring chamber (marker facing the screen) and replace the cap. **This needs to be done within <u>3 to 6 minutes</u> of adding the DPD reagent to the sample.**
- With the colourimeter on a flat surface, press the button on the meter with the check mark symbol. Read the total chlorine value (mg/L) on the digital screen and record the value in your field notebook.
- Also, record the date and the time when the water sample was collected and when the total and residual chlorine was measured, and the initials of the person who did the reading. Maintain a lab logbook to record the daily readings of total and residual chlorine (See Appendix VIII).
- When finished, dump the contents of the second sample cell into a waste bottle and thoroughly rinse the sample cell with deionized or distilled water.
- Note that the colourimeter records the most recent readings, plus the time when the readings were taken. These data can be accessed later to verify the field notes.
- Turn off the power on the meter.

All water samples collected after disinfection that give total chlorine values less than 2.5 mg/L and/or residual (free) chlorine values less than 0.5 mg/L should be reported to the facility manager.

APPENDIX I

A) IWEco report recommendations

1. St. Kitts Environmental Health Laboratory

- Turbidity meter
- pH meter
- Multi-parameter meter with probes capable of measuring salinity/conductivity and DO – for coastal water monitoring
- In-house equipment for external servicing: HACH DR3900 spectrophotometer

RESPONSE: Acquisition of a field turbidity meter (i.e., HACH 2100Q) and a field meter for analysis of pH, conductivity, salinity, TDS and water temperature (i.e., HACH Pocket Pro+ Multi-2) is recommended for the EH lab at St. Kitts. Acquisition of a HACH DR300 Pocket Colorimeter (Nitrate) is recommended for the EH lab in St. Kitts for analysis of nitrate. *In situ* analysis of dissolved oxygen in coastal areas is not recommended as a critical parameter for protection of human health. Also, DO probes require regular maintenance (e.g., changing membrane) and continuous calibration. As discussed previously in the report, service to the DR3900 spectrophotometer is recommended if a decision is made to analyze COD in wastewater samples.

2. **Nevis Water Department Laboratory**

1. Humidity/temperature monitor (hygrometer)
2. Thermometers for incubator x 2 and refrigerator x 2
3. Bench top spectrophotometer
4. Bench top conductivity meter

RESPONSE: The low-cost items such as a hygrometer and thermometers can be acquired by the WD lab in Nevis. This lab already has a HACH Pocket Pro+ Multi-2 meter for analysis of conductivity and other water quality parameters in the field and a Hach DR900 colorimeter for analysis of residual chlorine in the field. It is not clear why a bench top conductivity meter is also needed. Similarly, it is not clear why a bench top spectrophotometer is needed. Acquisitions of a HACH DR300 Pocket Colorimeter (nitrate) is recommended for the WD lab in Nevis for analysis of nitrate.

B) Needs for equipment and facilities identified by staff

1. Nevis Water Department

- New/refurbished cupboards and sink in the laboratory
- Microscopes (2)
- Back-up pump for water filtration (microbial testing)
- A refrigerator/freezer dedicated for laboratory use
- Bench-top spectrophotometer
- Bench-top conductivity meter
- Thermometers (for water bath and incubator)
- Distilled/deionized water generator
- Pick-up truck designated for water quality monitoring
- Larger laboratory space/building

RESPONSE: As discussed above, there does not appear to be justification for acquiring a bench top spectrophotometer and a bench top conductivity meter for the WD lab in Nevis. For analysis of nitrate, acquisition of a HACH DR300 Pocket Colorimeter (Nitrate) is recommended for the WD lab. A unit for producing deionized water and a designated refrigerator/freezer for storing samples are absolute requirements for the WD lab. If the lab switches to the IDEXX Colilert method, then microscopes and a backup pump for water filtration will not be necessary.

2. St. Kitts Department of Environmental Health

- Portable (field) multi-parameter meter and probe (pH, turbidity, salinity, conductivity, DO, etc.)
- Bench-top pH meter
- Bench-top turbidity meter
- Bench-top conductivity meter
- Microscopes (2)
- Back-up pump for water filtration (microbial testing)
- Bench-top spectrophotometer
- In-field infrared thermometers
- Pick-up truck designated for water quality monitoring
- Larger laboratory space/building

RESPONSE: Acquisitions of a field turbidity meter (i.e., HACH 2100Q) and a meter for analysis of pH, conductivity, salinity, TDS and water temperature in the field (i.e., HACH Pocket Pro+ Multi-2) and a colourimeter for analysis of nitrate (i.e., HACH DR300 Pocket Colorimeter – Nitrate) are recommended for the EH lab in St. Kitts. Therefore, there does not appear to be justification for acquiring a bench top turbidity meter, a bench top pH meter and a bench top conductivity meter for the EH lab. There also does not appear to be justification for acquiring infrared thermometers when the field meter can measure water temperature. *In situ* analysis of dissolved oxygen with a field meter is not considered a critical parameter for protection of human health. In addition, DO probes require regular maintenance (e.g., changing membrane) and continuous calibration. A designated refrigerator/freezer for storing samples is also recommended for the EH lab. If the lab switches to the IDEXX method for microbial testing, then microscopes and a backup pump for water filtration will not be necessary.

If a decision is made for the HD lab in St. Kitts to monitor the quality of treated wastewater, a vacuum pump will be needed for filtering wastewater for TSS analysis, so acquisition of a backup pump may be justified. Additional equipment required for TSS analysis includes a balance and a drying oven. For analysis of cBOD5, a dedicated incubator may be required for incubation of the BOD bottles at 20^{o}C for 5 days. As discussed in the report, service to the existing DR3900 bench top spectrophotometer is recommended if a decision is made to also analyze COD in wastewater samples.

GENERAL NOTES:

It is beyond the scope of the consultancy to make recommendations concerning improvements to laboratory space or the acquisition of field vehicles in both St. Kitts and Nevis. Acquisition of laptop computers for both the EH lab in St. Kitts and the WD lab in Nevis is recommended for direct data entry by technical staff.

APPENDIX II
DATA SHEET FOR WATER QUALITY ANALYSIS

Sample identifier (4 digits) _____ * Record in the comments section where (lab/field) and when the nitrate/nitrite analysis was done.

Site Identifier	Date Sample Collected	Time Sample Collected	Free Chlorine (mg/L)	Turbidity (NTU)	Salinity (mg/L)	Nitrate/Nitrite (mg/L)			pH	Cond (µS/cm)	TDS (mg/L)	Person Reporting	Comments/ Action Taken
						Test Strip		Colourimeter*	OPTIONAL DATA				

Reviewed by (*initial*): _____ Date: _____

APPENDIX III
DATA SHEET FOR MICROBIOLOGICAL ANALYSIS
Total Coliforms and E. coli

Sample identifier (4 digits) _____ *** Transcribe the free chlorine data from field notes.**

Site Identifier	Date Sample Collected	Time Sample Collected	Free Chlorine (mg/L)*	In Incubator		Out Incubator		Presence/ Absence		Quanti-Tray Results				Person Reporting	Comments/ Action Taken
				Date	Time	Date	Time	TC	E. coli	TC # wells	TC MPN	E. coli # wells	E. coli MPN		

Reviewed by (*initial*): _____ Date: _____

APPENDIX IV
DATA SHEET FOR MICROBIOLOGICAL ANALYSIS
Intestinal *Enterococci*

Sample identifier (4 digits) _____

Site Identifier	Date Sample Collected	Time Sample Collected	In Incubator		Out Incubator		Presence/ Absence	Quanti-Tray Results		Person Reporting	Comments/ Action Taken
			Date	Time	Date	Time		Enterococci # wells	Enterococci MPN		

Reviewed by (*initial*): _____ Date: _____

APPENDIX V
DATA SHEET FOR WASTEWATER ANALYSIS
Total Suspended Solids

Sample identifier (4 digits) _____

Site Identifier	Date Sample Collected	Aluminum Dish #	Initial Weight of Filter (g)	Sample Filtration			In Oven		Out Oven		Final Weight of Filter (g)	TSS Conc. (mg/L)	Person Reporting	Comments/ Action Taken
				Date	Time	Volume (mL)	Date	Time	Date	Time				
Blank						500 mL								

Reviewed by (*initial*): _____ Date: _____

APPENDIX VI
DATA SHEET FOR WASTEWATER ANALYSIS
Chemical Oxygen Demand

Sample identifier (4 digits) _____

Site Identifier	Date Sample Collected	Time Sample Collected	COD Concentration (mg/L)	Person Reporting	Comments/ Action Taken

Reviewed by (*initial*): _____ Date: _____

APPENDIX VII
DATA SHEET FOR WASTEWATER ANALYSIS
Biochemical Oxygen Demand

Sample identifier (4 digits) _____

Site Identifier	Date Sample Collected	Time Sample Collected	BOD Bottle #	Initial DO (mg/L)	In Incubator		Out Incubator		Final DO (mg/L)	Sample Dilution Decimal (e.g., 20% = 0.2)	$cBOD_5$ Conc. (mg/L)	Person Reporting	Comments/ Action Taken
					Date	Time	Date	Time					
Blank													

Reviewed by (*initial*): _____ Date: _____

APPENDIX VIII
DATA LOG FOR TOTAL AND RESIDUAL CHLORINE IN DRINKING WATER AFTER DISINFECTION

Location Identifier	Date Sample Collected	Time Sample Collected	Free Chlorine (mg/L)	Total Chlorine (mg/L	Conductivity (µS/cm) OPTIONAL	Person Reporting	Comments/ Action Taken

Reviewed by (*initial*): _____ Date: _____