Appendix A: Standard Operation Procedure
Field Sampling with Hand-held Instruments

Introduction

The mission of Hui O Ka Wai Ola is to generate quality-assured coastal water-quality data, and to provide this data to HIDOH, other resource agencies, non-governmental organizations, researchers and the public.

Specific goals of Hui O Ka Wai Ola are to 1) increase community capacity for long-term monitoring water quality in Maui coastal waters; 2) generate quality-assured reliable data that can be used to assess coastal water quality conditions and detect temporal trends that can augment HIDOH-CWB beach monitoring program sampling; 3) thereby empowering community and government mangers to take action to improve coastal water quality, benefiting the coral reef ecosystem and people alike.

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Chapter 1: Preparation

Safety

One of the most critical considerations for a citizen monitoring program is the safety of its volunteers. All volunteers are trained in safety procedures and should carry a set of safety instructions and the phone number of their monitoring team leader. Safety precautions cannot be overemphasized.

The following are some basic safety rules. At the site:

- Always monitor with at least one partner. Always let your monitoring team leader know where you are, when you intend to return, and what to do if you do not come back at the appointed time. Do not rely on cell phones, as a site may lack adequate reception.
- Know any important medical conditions of team members (e.g., heart conditions or allergic reactions to bee stings).
- Listen to weather reports. Never compromise your safety if severe weather is predicted or if a storm occurs while at the site.
- Use caution when entering the water. Never turn your back to the surf and waves. You should not be sampling in water greater than knee deep. If you are uncomfortable with the level of surf and are concerned for your safety do not go in the water! The most important thing is your safety. Data can be collected at another time.
- If you drive, park in a safe location. Ensure your car does not pose a hazard to other drivers and do not block traffic.
- Never cross private property without the permission of the landowner. For sites requiring access via private property, Hui O Ka Wai Ola will obtain permission for our volunteers but you may need to check-in before you monitor such a site.

Field equipment pickup

Before packing up the equipment, check the calibration log to ensure that all calibrations are up to date on the meters and the probes. In addition, make sure the battery power for each meter is sufficient for the field sampling session. If necessary, put new batteries in the meters and reset the date and time before leaving the regional laboratory.

In addition to the measurement equipment, collect supplies that may be required for collection and measurement of the in-situ water quality parameters (Chapter 3), for water quality filtration (Chapter 4) and for packaging samples for *Enterococcus* analysis (Chapter 5). The following list can serve as a guideline:

1. 1.5 gallon bucket
2. 100 ml bottles for turbidity samples
3. Distilled water
4. Field guide and equipment user manuals
5. Field notebook
6. Data sheets and clipboard
7. Chain of custody forms
8. Cooler with blue ice
9. Ziplocks for chain of custody forms
10. Extra batteries for meters
11. Pens for filling in data sheets, sharpies for writing labels
12. Label tape
13. Kim-wipes and paper towels
14. Gloves

15. Scissors 16. Camera

The following equipment is necessary for **in-situ measurements**:

1. Digital thermometer
2. Hach 2100Q turbidimeter
3. Hach HQ40d meter with the following electrodes:
    a. IntelliCAL LDO101 DO sensor
    b. IntelliCAL CDC401 conductivity probe
    c. IntelliCAL PHC101 pH electrode

The following equipment is necessary for **field water sample collection for nutrient sampling**:

1. Sample bottles (clear, HDPE, 125mL, acid-washed)
2. Filters (GF/F, 25mm)
3. 60-mL syringe with luer locks

The following equipment is necessary for collecting bacterial information with the Enterolert system:

1. Whirlpack bags
2. Gloves

Labeling

Any samples that will be brought back to the local laboratory either for testing or shipping to an analysis lab must be labeled. It is always best to label bottles and samples *before* you get to the field, if possible. Hui O Ka Wai Ola has a strict labeling scheme to prevent sample mix-ups.

Each sample collected will be labeled with the following information prior to or during the collection of the sample:

a. a unique sample number,
b. sample type,
c. name of collector,
d. date and time of collection, and
e. place of collection

The sample number will follow this code: 3-letter site location code, two-digit year, two-digit month, two-digit day – sample type code (N for nutrients, S for suspended sediment) – sample number. Letters are used for sample duplicates. For instance, a sample at Honokowai Beach Park might be: HBP150601-N-1. The initials of the sampler will be listed separate from the sample ID.

Observations

On the field data sheet, include observations including the tidal information, time of day, wind speed (see Table 1) and direction and wave state. Note the moon phase if possible, and the number of swimmers in the water near the sample site.

Table 0-1: Beaufort wind scale

Estimating Wind Speed			
knots		**Effects Observed at Sea**	**Effects Observed on Land**
under 1	calm	Sea like a mirror	Calm; smoke rises vertically
1-3	light air	Ripples with appearance of scales; no foam crests	Smoke drift indicates wind direction; vanes do not move
4-6	light breeze	Small wavelets; crests of glassy appearance, not breaking	Wind felt on face; leaves rustle; vanes begin to move
7-10	gentle breeze	Large wavelets; crests begin to break, scattered whitecaps	Leaves and small twigs in constant motion; light flags extended
11-16	moderate breeze	Small waves 2-4 feet high, becoming longer; numerous whitecaps	Dust, leaves, and loose paper raised up; small branches move
17-21	fresh breeze	Moderate waves 4-8 feet high taking longer form; many whitecaps; some spray	Small trees in leaf begin to sway
22-27	strong breeze	Larger waves 8-13 feet high forming; whitecaps everywhere; more spray	Larger branches of trees in motion; whistling heard in wires

Chapter 2: Calibration

PRINCIPLE:

Perform any required standards checks before leaving the regional laboratory, and note the date, time and calibration parameters in the field notebook AND the chain-of-custody form. The following instruments should be calibrated, as noted:

Instrument	Parameter	Schedule	Field-check acceptance criteria
NSIT-traceable waterproof digital thermometer	Temperature	None (factory-calibrated)	None
Hach HQ40d meter, IntelliCAL CDC401 conductivity probe	Salinity/ conductivity	Quarterly or as needed	± 5% of calibration solution
Hach HQ40d meter, IntelliCAL LDO101 luminescent/optical DO sensor	Dissolved oxygen	Quarterly	Post-check ± 5 % of pre-check
Hach HQ40d meter, IntelliCAL PHC101 pH Electrode	pH	Monthly	± 5 % of calibration solution
Hach 2100Q turbidometer	Turbidity	Yearly or as needed	± 5 % of Gelex standards (5, 50, 500 NTU). Deionized/turbidity-free bank < 0.25 NTU

The Hach 2100Q turbidimeter should be checked with the secondary Gelex standards before and after the field session.

METHODS:

Turbidimeter calibration check

1. Use the Gelex secondary standards to perform QC check of the turbidimeter.
2. Handle the Gelex standards by the lid. Avoid touching the sides of the glass vial.
3. Power the turbidimeter on, insert the calibrated Gelex standard into the well, close the door on the sample cell, and push the READ button.
4. Record the reading on the data sheet for that particular Gelex standard.
5. Repeat the process with all three of the Gelex standards.
6. Insert a sample cell with distilled water into the turbidimeter and take a reading of the distilled water to use as a field blank. Record this reading on the data sheet.

Hach HQ40d meter and probes

1. Use the check standards on the pH (page 49 in the user's manual) and conductivity (page 65 in the user's manual) probes to verify the probes are within standards before leaving the regional laboratory.

Chapter 3: Water collection for in-situ measurements

PRINCIPLE:

It is important that the water collection for in situ measurements happens at the *same place, same time* and from the *same pool of water* each time.

MATERIALS AND EQUIPMENT

- 1.5 gallon bucket
- Sample bottles

METHODS:

1. Submerge the bucket/bottle 6 to 12 inches below the surface facing into the oncoming waves.

2. Cap the turbidity bottle while it is still under the water.

PROCEDURAL NOTES:

General sampling techniques for in-situ measurements:

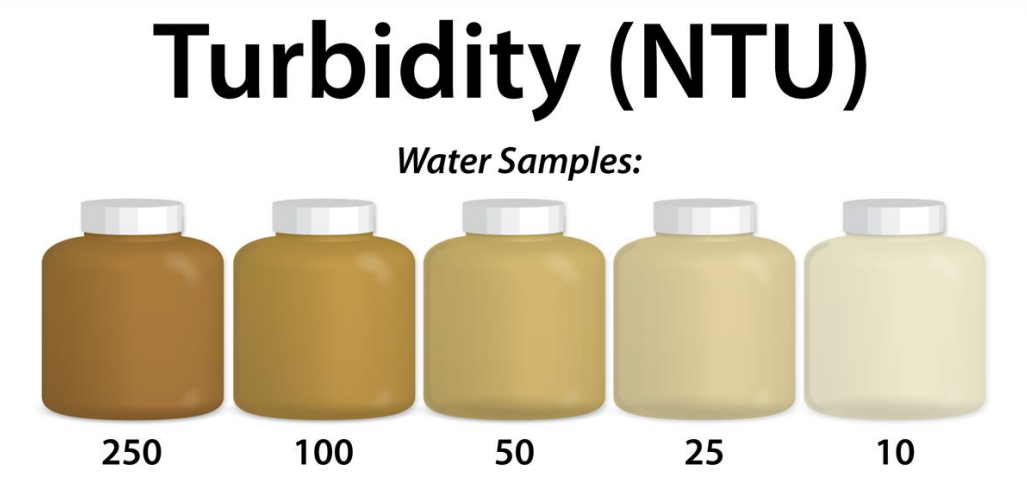
Because it can be hazardous to stand in the ocean where the surf is breaking while attempting to use a hand-held meter, water for four of the in-situ measurements ***will be collected in a 1.5 gallon bucket and taken back away from the ocean to conduct the measurements for temperature, salinity, pH, and dissolved oxygen*.** Water for the turbidity measurements will be collected in a smaller bottle that can be re-agitated to provide the most accurate turbidity measurements.

In general, you should always collect water samples with the water moving towards you. Always face away from the shoreline at a depth no more than knee deep and rinse the bucket/turbidity bottle three times with the water to be tested. When collecting the sample, avoid disturbing any silt that may have settled on the bottom.

Measurements on water samples should be made in a shady area if possible, avoiding direct sunlight. The samples should be tested as quickly as possible once the water is removed from the water body being sampled.

Turbidity measurements

A turbidity meter consists of a light source that illuminates a water sample and a photoelectric cell that measures the intensity of light scattered at a 90 degree angle by the particles in the sample. It measures turbidity in nephelometric turbidity units (NTU). The meter can measure turbidity over a wide range from 0 to 1000 NTUs. These values can jump into hundreds of NTUs during runoff or flood events.



MATERIALS AND EQUIPMENT:

- Hach 2100Q turbidimeter
- Distilled water in squirt bottle
- Cloth to clean sample bottles

METHODS:

3. Review the general techniques for turbidity measurements in the turbidimeter user guide as required. Pay particular attention to handling of the sample cell to avoid compromising the measurements.

4. Empty the distilled water from a clean sample cell.

5. Gently agitate the water sample to be tested to ensure that any sediment that may have fallen out of suspension is re-suspended in the sample.

6. Rinse the clean sample cell 3 times with the water sample, taking care to handle the sample cell by the top of the cell to avoid getting fingerprints on the sample cell glass.

7. Fill the sample cell to the line taking with the water to be sampled.

8. Insert the sample cell into the turbidimeter sample compartment with the arrows lined up on the cell and the meter.

9. Close the instrument sample compartment door.

10. Make sure the instrument is measuring in NTUs and averaging is on.

11. Press the READ button and record the reading on the data sheet.

12. Remove the sample cell from the instrument compartment and discard the water sample. Rinse the sample cell 3 times with distilled water, taking care to avoid getting water on the outside of the sample cell. Any excess water can be gently blotted with a Kim-wipe cloth. Fill the sample cell to the line with distilled water and test the distilled water to ensure the sample cell is clean. The reading should be < 0.1 NTU. If necessary, repeat the rinse and re-test until the cell is clean. Sample cells should be stored with distilled water.

PROCEDURAL NOTES:

- The turbidimeter (Hach 2100Q) should be placed on a dry flat surface while making measurements.

Grab samples:

How to collect a "grab" sample for turbidity

- If needed, label the bottle with the site number, date, time and your name or initials. Use waterproof pen.
- Remove the cap from the bottle just before sampling. Avoid touching the inside of the bottle or the cap. In high flows, use a sampling pole. Rinse the sampling bottle on the pole 3 times prior to decanting water into sample bottle.
- It is best to collect samples while standing on a rock. If you need to wade, try to disturb as little bottom sediment as possible. Be careful not to collect water that contains bottom sediment. Collect the water sample in front of you (towards the ocean).
- Hold the bottle near its base and immerse it (opening upwards) below the water surface. Collect a water sample 6 to 12 inches (~0.3m) beneath the surface or mid-way between the surface and the bottom if the water level is shallow.
- Turn the bottle underwater into the current and away from you in an upstream direction. Fill the bottle completely and make sure there is no headspace in the container.
- Check off the test on your appropriate field data sheet and record the time. This is important because it tells the monitoring coordinator that this sample has been collected from your site.
- The hold time for turbidity is 24 hours

Salinity (Conductivity), pH and Dissolved Oxygen

PRINCIPLE:

Salinity (Conductivity)

Salinity is a key factor affecting the physical make-up of an estuary, and is defined as the concentration of dissolved salts in the water, usually expressed in parts of salt per thousand parts of water (ppt). Seawater averages 35 ppt (3.5% by weight) in the open ocean and 27 to 33 ppt (2.7 to 3.3% by weight) in coastal waters. Fresh water contains few salts - drinking water usually has a salinity of less than 0.5 ppt. A liter of Casco Bay water would typically contain 28 to 34 grams of dissolved salts. In other words, a quart would contain about an ounce of salts.

The surface salinity levels within the Bay, especially near the coast, vary with many factors, including the tides and the volume of fresh water flowing into the Bay. Salinity tends to decrease in the spring when heavy rainfall, the release of groundwater, and melting snow combine to greatly increase the amount of fresh water flowing in. In late summer and fall, particularly during periods of drought, higher levels of salinity may extend farther up some reaches of the estuary as the fresh water flow decreases. Some decreases in salinity can be attributed to human activities which reduce the water-holding capacity of the land (such as paving or removal of vegetation) or directly accelerate fresh water discharge (such as storm sewers). On the other hand, excessive withdrawals of water from the fresh water portion of a tributary (for agricultural use, drinking water, etc.) can elevate salinity near the mouth of this tributary.

Salinity levels also vary vertically from top to bottom. In general, salinity increases with depth. The fresh water coming down river is less dense than the heavier seawater, so the entering fresh water tends to float on top of the seawater and may not mix immediately. The volume of entering fresh water is also the greatest closest to land. The net result is a wedge of lighter fresh water lying over the heavier seawater, with poorly defined edges that are continually mixed by wind, waves, and tides. In shallow waters, the mixing of top and bottom layers can obscure this "wedge" completely.

Dissolved Oxygen

Dissolved oxygen (DO) is one of the most important indicators of the quality of water for aquatic life. It is essential for the basic metabolic processes of animals and plants inhabiting our coastal waters. Dissolved oxygen is measured in milligrams per liter (mg L^{-1}). When oxygen levels fall below about 3 to 5 mg L^{-1}, fish and many other marine organisms are stressed and some can not survive. Dissolved oxygen is a particularly sensitive constituent because other chemicals present in the water, certain biological processes, and physical factors such as temperature and water clarity exert a major influence on its availability throughout the year.

The maximum amount of oxygen water can hold depends a great deal on its temperature and salinity. A DO test (using a meter or chemical kit) tells you how much oxygen is dissolved in the water, but it does not tell you how much oxygen the water is capable of holding at the temperature and salinity at which it was tested. Warmer water holds less dissolved oxygen; as water approaches its boiling point, it can hold almost no oxygen. Dissolved oxygen also decreases with increasing salinity. When water holds all the dissolved oxygen that it can at a given temperature and salinity, it is said to be 100 percent saturated with oxygen. If water holds

only half that amount of DO at the same temperature and salinity, it is said to be 50 percent saturated. The table below shows this relationship for various temperatures and salinities.

Table 0-1: Potential dissolved oxygen levels in milligrams per liter (mg/l) at sea level

TEMPERATURE	SALINITY			
°C	FRESH WATER 0 PPT	BRACKISH WATER 5 PPT	NEARSHORE WATER 32 PPT	OPEN OCEAN 35 PPT
0	14.6	14.1	11.6	11.3
5	12.8	12.4	10.3	10.1
10	11.3	11.0	9.2	9.0
15	10.2	9.9	8.4	8.3
20	9.2	9.0	7.6	7.5
25	8.4	8.2	7.0	6.9
30	7.6	7.4	6.2	6.1

pH

pH is a measure of how acidic or basic a solution is. Pure distilled water has a pH of 7.0 and is said to be neutral - but pure distilled water is rarely found in nature. The pH values of natural waters are controlled by the salts and gases dissolved in them. Seawater typically has a pH of 8.1 to 8.3. Because its pH is greater than 7.0, it is said to be basic or alkaline (the two terms are synonymous). The pH of seawater is fairly stable because it's highly buffered - that is, the water contains pairs of ions which react to damp down changes in pH (for more information on buffers, see the box on page 19).

The strong buffering and constant motion of seawater tend to minimize variations in pH. Short-lived, local variations may be caused by intense phytoplank-ton blooms, or at locations where industrial discharges and sewer outflows enter the ocean, or where there are large influxes of fresh water. Natural fresh water typically has a lower pH than seawater. Rain water usually has a pH of 5.6 to 5.8. Because its pH is less than 7.0, even unpolluted rain water is said to be acidic. So-called "acid rain" has an even lower pH due to atmospheric pollutants.

pH is defined as the negative logarithm of the concentration of hydrogen ions; the higher the concentration, the lower the pH. In any given aqueous solution, a certain proportion of water molecules dissociate to form hydrogen (H+) and hydroxyl (OH-) ions:

$$H2O \rightarrow H^{+} + OH^{-}$$

MATERIALS AND EQUIPMENT

- Hach HQ40D probe

METHODS:

Before attaching any probes, make sure the date and time are set correctly on the Hach HQ40d meter. If the date and time are not set properly before the probes are attached for the first time, the probes will retain the incorrect date and time for the remainder of their service lives. Therefore it is essential that the date and time be checked before starting to use the meter. Without connecting the probes to the meter, place all 3 probes in the bucket with the collected water sample so that the probes come to the ambient temperature of the sample. Allow the probes to sit in the water for at least 5 minutes before connecting them to the meter to take measurements of the sample. While the probes are soaking in the sample, the turbidity measurements can be made.

Review the instructions in the users manual for the hand-held meter and data probes as required.

1. Two probes at a time can be attached to the meter. **Begin with the pH and conductivity/salinity probes.**

2. Switch the meter display so that the pH probe data i**s displayed. To measure the pH, place the probe in** the sample and press the GREEN/RIGHT key under Read. Once the measurement has stabilized, the lock icon will appear and you can record the measurement on the data sheet.

3. Simultaneously, the conductivity probe can be placed in the sample. Once the pH reading has stabilized switch the meter to display the conductivity probe. To measure salinity, press the GREEN/RIGHT key under Read. When the measurement has stabilized, the lock icon will appear and you can record the measurement on the data sheet.

4. Remove the probes from the water sample and rinse the probe with distilled or de-ionized water. Blot the probes with a Kimwipe to remove any remaining water droplets. Place the pH probe back in the 3M KCl solution vial. Make sure to wipe any water that might have gotten on the meter off and store the meter and probes back in their case for transport.

5. **Be sure to log your results both in the field notebook**

Water temperature

PRINCIPLE:

Water temperature in Hawaii fluctuates with the season, as shown in Table 2 (in Fahrenheit). Given the frequency of bleaching events in 2015 and 2016, collecting water temperature can help track localized variations between sites.

Table 2: Water Temperature Table of the Hawaiian Island Coast

Location	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
Honolulu Oahu Island	76	76	76	78	79	80	80	81
Hilo Hawaii Island	72	71	72	72	74	74	75	75
Kahului Maui Island	75	75	76	78	79	79	80	80
Kawaihae Hawaii Island	77	77	78	79	80	81	81	80
Mokuoloe, Oahu Island	74	74	75	77	79	79	80	80
Nawiliwili Kauai Island	77	77	78	79	81	82	82	83

MATERIALS:

- Hach WQ40D
- Digital thermometer

METHODS:

1. **Presoak:** Presoak the thermometer with 1” of water, in the shade, upright in a container for at least 10 minutes, with the power off. Be sure the water level stays below the digital display on the thermometer.

2. **Take measurements**: Preferably dip the thermometer into the water. If the sample is being taken directly from the water, stand so that a shadow is cast upon the site for temperature measurement. If you are using a sampling arm, acquire a fresh sample of river water, stand in the shade, and stir gently. The thermometer should be held by its top and immersed into the water. Allow the thermometer to stabilize for at least one minute, then without removing the thermometer from the water, read the temperature to the nearest 0.1°Cand record.

3. The digital thermometer should be used for quality control of the Hach temperature meter, and follow the same process described above.

PROCEDURAL NOTES:

Note: It is best to begin soaking the thermometer in and leave it while the rest of the measurements are taken – up to 10 to 15 minutes – to get an accurate reading. If one waits longer, the water will equilibrate with the air temperature.

Keep the bucket out of the sun to more accurately measure temperature.

Data quality

When to take a 4th measurement:

- Temperature: If there is a difference of 1ºC or greater between any of your three measurements
- pH: If there is a difference of 0.2 or greater between any of your three measurements
- Conductivity: If there is a difference of greater than 10 uS between any of your three measurements
- Dissolved Oxygen: If there is a difference of 0.4 ppm or greater between any of your three measurements
- Turbidity: If there is a difference of 0.2 NTU or greater between any of your three measurements

Chapter 4: Nutrient sample processing

PRINCIPLE:

"Nutrients" describe nitrogen and phosphorus-based compounds that are used by microalgae and bacteria for growth. The coastal waters of Hawaii are considered to be nutrient-limited – meaning that nutrients are the limiting factor for the growth of phytoplankton. Measuring nutrient concentrations accurately can help to track potential sources of nutrients.

Nutrients in Hawaii are often sorbed on to sediments, so it is important to filter the sample to remove the potential for sediments to bind to nutrients. For this reason, we also use acid washed bottles in order to minimize binding with the side walls of the bottle.

MATERIALS AND EQUIPMENT

- 125 mL HDPE acid-washed sample bottle
- In laboratory or field filtration equipment, including filter, filter forceps, filter housing and vacuum device.

METHODS:

1. Collect a sample from the wash zone (approximately knee height) in the 125 mL HDPE sample bottles, as directed above.

Filter the sample into the bottle being used for water quality (nutrient) analysis.

2. Hook up a 60-mL syringe to a prepared Swinx filter housing with filter.

3. Prepare an acid-washed 125-mL HDPE bottle to collect the filtrate into.

4. Place a filter in the filter housing using forceps (this can either be a Nalgene 500mL rig or a 25mm Swinnex holder combined with a 60-mL syringe.

For Nalgene rig:

5. Pour ~ 250 mL of the sample water into the reservoir atop the filter rig, and begin pumping until vacuum occurs and flow is continuous. Vacuum pumping can either be with a hand pump or with a vacuum pump. In either case, make sure that the vacuum line is attached to the filter housing.

6. Make sure that the seal is working. Add an additional 250 mL until the total 500 mL sample is processed. Collect ~80mL of the filtrate (bottom part) into the 125 mL bottle. Label the sample as described in Chapter 1.

7. Once the entire 50 mL volume has passed through the filter, disassemble the filter rig

For Swinnex hand filtration:

8. Place a filter (white, not blue paper) in the Swinnex with forceps. Be sure that the o-rings are in place. Use a smalled spray of DI water to make sure the filter stays in place.

9. Remove the plunger for a 60mL syringe, and attached the Swinnex on the syringe. Carefully pour 50mL of water to be filtered in the syringe. Use the plunger to filter the water into a 125mL bottle.

If particulate N and C are to be analyzed:

a. Using a set of tweezers, carefully fold the 0.2um filter and place in a folded up piece of aluminum foil. The foil can then be placed in a plastic filter holder or Ziploc bag for labeling.

b. Label the filter with P for particulate N and particulate C. Label the filtrate (the water that has been filtered) using N for the sample type code.

Store the sample:

10. Store the nutrient sample upright at -20°C in a cooler with wet or blue ice – after frozen it does not need to be upright anymore.

11. Store the filter and sample once back in the regional laboratory in a freezer kept at -20°C.

12. Record the temperature of the water and other specifications in the log book and on the chain of custody form. Copy the salinity from the in-situ measurement and volume of the sample.

PROCEDURAL NOTES:

- The above procedure can be done in the field or in the lab, but the lab may be a more controlled environment and easier to work in.
- Be careful of changing water conditions when collecting sample. Wear gloves to prevent contamination.
- To collect the sample water for nutrient analysis use a 1-L HDPE bottle that you can pour into smaller bottles.
- For suspended sediment, use a 500-mL HDPE bottle.
- Remove the cap from the bottle just before sampling.
- Avoid touching the inside of the bottle or the cap.
- In high flows, use a sampling pole.
- Rinse the sampling bottle on the pole 3 times prior to decanting water into sample bottle.
- Collect the sample from wading depth. Try to disturb as little bottom sediment as possible. Be careful not to collect water that contains bottom sediment. Stand facing upstream. Collect the water sample in front of you (upstream).
- Hold the bottle near its base and immerse it (opening upwards) below the water surface. Collect a water sample 6 to 12 inches beneath the surface or mid-way between the surface and the bottom if the stream reach is shallow.
- Turn the bottle underwater into the current and away from you in an upstream direction. Fill the bottle completely and make sure there is no headspace in the container.

QUALITY CONTROL PROCEDURES

Chapter 5: Suspended-sediment sample processing

PRINCIPLE:

Sediments act as both primary and secondary pollutants in the coastal environment. Sediments, especially fine sediments, can reduce water clarity and reduce the amount of light that is available for photosynthesis in coral reef ecosystems.

The suspended sediment concentration (SSC) method described here accounts for both terrestrial and marine-originated sediments. SSC in the coastal zone can also include suspended solids of biological origin.

The reason we are interested in suspended sediment as a variable is that we want to correlate the turbidity measurements we are doing with field instruments in real time with laboratory data.

MATERIALS AND EQUIPMENT:

- 500 mL filtration units
- 47 mm filters (GF/F)
- Drying oven at 60°C
- Analytical balance
- Filter forceps
- 47mm petri dishes
- Aluminum foil

PROCEDURE:

1. Collect the sample as described above for nutrient analysis. For suspended sediment, use a 500-mL or 1000-mL HDPE bottle.

2. After the sample is collected, store the sample on ice until the sample is either

    a. Shipped to S-LABS for analysis or

    b. Analyzed in a satellite lab on Maui.

    The procedure below describes the protocol for all laboratories.

Prepare the filters:

3. Pre-weigh a 47 mm GF/F filter using the analytical balance. Weigh each filter three times, and record each value.

4. Place the pre-weighed filter in its own 47 mm petri dish or similar plastic container.

5. Write the average of the three values on the cover of the petri dish.

Filter the sample:

6. Use the filter forceps to place a pre-weighed 45um filter on the filter pad of the filtration unit.

7. Use a small drop of deionized water to wet the filter.

8. Secure the unit together by twisting the top on to the bottom of the unit.

9. Attach the vacuum tubing to the unit and turn on the vacuum. Listen or look for possible leaks.

10. Pour 500 mL of the sample into the top portion of the rig. Use the vacuum pump to create suction. If there is an additional 500 mL, empty the bottom part of the rig into the sink (this is waste) and use the same filter to filter the next 500 mL.

11. Turn off the vacuum and release the pressure slowly by opening one of the small valves.

12. Using forceps, collect the filter in an aluminum foil piece by folding it first in quarters. Write the sample number of the foil using the special pen that can go into the oven.

Dry the sample in the oven:

13. Store the aluminum foil package in a plastic container for filters.

PROCEDURE NOTES

- Gloves are recommended to maintain the quality of the samples.
- If the vacuum is not suctioning correctly, check the various o-rings that are part of the filtration units.

QUALITY CONTROL PROCEDURES

REFERENCES

Standard Methods for Examination of Water and Wastewater, 18th Edition, APHA.

Chapter 6: *Enterococcus* sample collection and measurement using the IDEXX Enterolert and Quanti-tray system

PRINCIPLE:

Importance of Bacteria
Enterococcus bacteria are generally not harmful by themselves but do indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems. Elevated levels of these bacteria can cause health problems (including ear infections, stomach upset and urinary tract infections in women), cloudy water, unpleasant odors, and an increased oxygen demand (the amount of oxygen consumed by microorganisms in breaking down waste). The EPA recommends *Enterococcus* as an indicator of health risk from water contact in recreational waters.

MATERIALS:

- Sterile 100 mL sample bottle or whirlpack
- Cooler with blue ice
- Enterolert™ (Idexx) reagent snap-packs
- 90 mL sterile DI water blanks
- Quanti-Tray 51-well trays
- Quanti-Tray heat sealer

METHOD:

Collect a Bacteria Sample:

1. Label the bottle or Whirlpack with the site number, date, time and your name.

2. Remove the cap from the bottle just before sampling. Avoid touching the inside of the bottle or the cap. If you accidentally touch the inside of the bottle, please report it to the monitoring coordinator and write it on data sheet. Our sterile bottles sometimes contain a pellet of sodium thiosulfate. This is for tap water samples, not river water samples. It can be left inside. Its presence is not important.

3. Wade in and try not to disturb the bottom or collect water with bottom sediment. Stand facing the water, or collect while kneeling on a rock.

4. Hold the bottle near its base and immerse it into the vertical water column with the opening upward. Collect a water sample 8 to 12 inches beneath the surface or mid-way between the surface and the bottom if the stream reach is shallow.

5. Turn the bottle underwater into the current and away from you in an upstream direction.

6. Fill to the black line. Do not fill the bottle completely so that the sample can be shaken just before analysis. Recap the bottle carefully, remembering not to touch the inside.

7. If you are assigned a WhirlPak, be sure it is labeled and wear gloves.

8. Tear off the top seal on the perforated line. Pull the short White tabs to open the pak; tightly grab the yellow tabs and fill the pak with river water facing upstream, in the middle of the water column. It must be filled to the 4 oz line.

9. Holding the yellow tabs, spin the pak away from you to close the opening, pull tight and TWIST the yellow tabs tightly to seal the pak. Hold the pak upside down and squeeze gently, NO WATER should leak out.

10. Store the pak upright in the cooler to avoid leaking.

11. Indicate bacteria collected on your field data sheet with the time collected.

12. Place samples in the cooler with blue ice for transport to the local Maui lab.

Bacterial Sample Processing with Enterolert:

1. Turn on IDEXX Quantitray sealer. Allow about 10 minutes for it to warm-up. Sealer is ready when the green light is lit on the front of the sealer.

2. Check the incubator temperature and adjust if not 41°C ± 0.5°C

3. Wash hands with soap and water and don powder-free disposable gloves for protection.

4. Sterilize laboratory working surface with isopropyl alcohol.

5. Place unopened seawater sample bottles or cups with unopened WhirlPaks on work surface. Be sure each sample container is marked with the sample source location. (Samples must be no older than 6 hours and must have been stored on ice.)

6. Place unopened 100 mL sterile sample bottles on work surface and label each with the corresponding sample source location.

7. Remove the correct number of Enterolert media packets from refrigerator storage and place on the work surface.

8. Process each sample and corresponding laboratory data sheet separately.

9. Fit a new sterile pipette to the pipette gun. Open a seawater sample container and transfer 10 ml of sample water to the corresponding sterile 100 ml sample bottle to make a 1:10 dilution. (*If you must put the bottle cap down, place it open side down on the sterile work surface.*) Place the pipette on the work surface in case you have to make a second attempt with THIS sample.

10. Add 90 ml of distilled or deionized water to the sample bottle. (Fill to the 100 ml line.)

11. Take one Enterolert packet, tap it to settle contents, and snap it open away from you. (*Do not breathe any Enterolert dust.*) Add the contents to the sample bottle, being careful not to insert your fingers or the packet into the bottle.

12. Replace the bottle cap and gently swirl the bottle until the Enterolert dissolves. (*About one minute.*) Do not shake the bottle and make bubbles.

13. Mark a Quantitray with the following information. Use a marking pen to avoid puncturing the fragile Quantitray backing. (*If this step is performed in advance for all of the samples, be sure to match the sample with the proper Quantitray.*)

    - Sampler's name
    - Tester's name
    - Date
    - Time collected
    - Sample site
    - Time into incubator
    - Time out of incubator (Next day)

    Results next day:

    - Number of positive small wells
    - Number of positive large wells

    MPN * number from IDEXX MPN Table 10 x MPN

    * MPN = Most Probable Number of Colony Forming Units

14. After the Enterolert has dissolved, open the sample bottle. Pick up the appropriate Quantitray in one hand and gently bow it to form a gap between the cells and the backing. The backing tab may be used to assist this, but do not insert fingers into the Quantitray. (*If the backing rips, it will not seal.*) Gently pour the sample into the Quantitray. Gently tap the Quantitray to remove any bubbles.

15. Place the Quantitray on top of the sealer's orange rubber mat. Run the mat and Quantitray through the sealer with the small wells first.

16. If the Quantitray seals without damage, discard the pipette and the remaining seawater sample, and return to **step 9** for the next sample, if any.

17. Place the sealed Quantitray(s) in the 41°C ± 0.5°C incubator for 24 hours (28 hours maximum). At this point, the Quantitrays are biohazardous material.

Clean up:

18. Clean the work area and dispose of gloves, sample bottles, pipettes, and empty packets in regular trash. Turn off the Quantitray sealer. (Note: Pipettes can be autoclaved and reused.

19. Remove the Quantitrays from the incubator and read each one with an ultraviolet lamp. Mark each positive (blue) cell with a marking pen and record the number of small and large positive cells.

Read the data after 24-28 hours:

20. Read the results by placing a 6-W, 365 nm wavelength UV light within 5 in of the tray in a darkened environment. Blue fluourescence indicates the presence of enterococci.

21. Read the IDEXX MPN Table by going across to the right by the number of positive small cells and down by the number of positive large cells. Record this number as the MPN.

22. Multiply by 10 the MPN just entered (to account for the dilution). This is the real MPN because you diluted the sample during preparation. Record the date and time read.

23. Transfer all of the data from each Quantitray to the corresponding laboratory data sheet.

Clean up:

24. Place used Quantitrays in standard red biohazard bags for autoclaving and disposal.

25. Ensure that the work area is clean and the ultraviolet light is turned off.

PROCEDURE NOTES:

- Wearing gloves is mandatory to prevent sample contamination and for your safety for either container.
- Be sure that the UV light is facing away from your eyes and towards the tray.
- If the sample is inadvertently incubated over 28 hours with out observation, the following guidelines apply: Lack of fluorescence after 28 hours is a valid negative. Fluorescence after 28 hours is an invalid test result.

QUALITY ASSURANCE:

The following QC should be performed on each lot of Enterolert reagent. Organisms used for Enterolert QC:

- *Enterococcus faecalis* ATCC 29212
- *Enterococcus faecium* ATCC 35667

- *Serratia marcescens* ATCC 8100
- *Aerococcus viridans* ATCC 11563

Chapter 7: Shipping and Handling

Sample delivery to the laboratory:

All samples collected in the field should be put on ice in the cooler as soon as they are collected. These samples should be brought to the local laboratory as soon as all of the assigned sample sites have been tested for the day. If enterococcus samples have been collected, they must be brought to the local laboratory for testing as soon as possible. The total time between collection and testing of enterococcus should not exceed 6 hours. Samples collected for nutrient analysis should also be stored on ice as soon as they are collected and filtered. Nutrient samples will be frozen once they are brought to the local laboratory until they are shipped to the analysis laboratory. Samples collected for suspended sediment will be processed at the local laboratory and should also be kept on ice until brought to the local laboratory.

The regional team leader is responsible for shipping nutrient samples to the analysis laboratory. Samples will be shipped to the S-Lab within 14 days of collection to ensure they are received and analyzed within the specified holding times for each analyte. The team leader will include the shipping number on the chain of custody form when the samples are shipped. Samples will be shipped frozen with blue ice in coolers to preserve the samples for analysis.

Receipt and logging of sample:

In the laboratory, the sample custodian inspects the condition and seal of the sample and reconciles label information and seal against the chain of custody record before the sample is accepted for analysis. After acceptance, the custodian assigns a laboratory number, logs sample in the laboratory log book and stores it in a secured storage room or cabinet or refrigerator at the specified temperature until it is ready for analysis. Once the sample is in the laboratory, the supervisor or analyst is responsible for its care and custody.

Disposal: Hold samples for the prescribed amount of time for the project or until the data have been reviewed and accepted. Document the disposition of samples. Ensure that disposal is in accordance with local, state, and U.S. EPA approved methods.