Bight ’08 Protocols for Collecting and Processing Sand, Seawrack and Concrete Coupons
May 3 2010

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Contact Information

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Field Sample Collection

Field Equipment and Supplies

Chain of custody/field sampling sheet
Clip board, pens and sharpies
Orange vests
Boots/waders
Orange street cones (if needed for traffic safety)
1L pp bottles, sterile (for water: 1 for each sample)
50 cc sterile centrifuge tubes (for sand: 2 for each sample)
Zip-lock bags (for sea wrack: 1 for each sample)
Paper towels
Gloves
Waste bag for gloves, paper towels, etc.
Coolers with blue ice packs
Hand sanitizer
First Aid Kit
Camera

Additional Items for Coupon Study

Portable pH/conductivity/temp/DO meter
Turbidometer
Wire cutter (to cut coupons)
PBS (phosphate buffered saline)
2L polypropylene (pp) bottles, sterile (pre-filled with 500 ml PBS)
Field equipment to measure flow rates & velocity

Sample Types and Location

Note: effort will be made to try to collect during neap tide.
On an outgoing tide, collect the following samples:

- Water sample at the interface of the storm drain/river/creek and the beach (Sample W_0);
- Water sample upstream of the drain/river/creek that is above tidal influence in the drainage system (Sample W_1);
- Sand sample 5 yards downstream* of W_0 (Sample S_5);
- Sand sample 25 yards downstream* of W_0 (Sample S_{25});
- Water sample 25 yards downstream* of W_0 (Sample W_{25});
- Sea wrack sample between W_0 and S_{25} at the mean high tide line (Sample K).
  *Downstream will be determined at the time of sampling by the beach sampler by observation of flow. Sample will be collected at high neap tide line.
- [Optional] Sand sample directly underneath sea wrack (Sample S_k) -- No need to process, just freeze once taken back to lab.
  [Optional] Coupon sample** (Sample C)
  [Optional] Sand/Biofilm EPS Sample for Ngoc Hoang at UCSB Lab

Sample Collection

1. Wear gloves for all sampling, change gloves between samples
2. Fill out field recording sheet at the site (optional: take photos to document field observations).
3. Check to make sure all sample containers are correctly labeled.
4. For **water** (Sample W0, W1, W25), fill 1 liter bottle, cap, and place in cooler.
5. For **sand** (Sample S5, S25, Sk*)
    1) Collect by scooping the top layer (1 inch deep) using the 50ml centrifuge tube. Try to scoop from multiple locations within a 0.5~1 meter diameter. Two near-full tubes will give ~100g. Avoid collecting sand near bird feces.
    2) Decant excess water and cap securely, place in cooler.
    * **Note: Sample Sk is optional**. Just use the same technique to collect two tubes *under the sea wrack* (sample K, see below). These samples will be processed by Ngoc Hoang at UCSB for biofilm and EPS. Store at -20C until end of study.?
6. For **sea wrack** (Sample K)
    1) Collect approx. 75 - 100 g of wrack (handful) stranded on sand between W_0 and S_{25} at the mean high tide line.
    2) Avoid fresh wrack (i.e., recently washed up on beach), and wrack that is too decomposed or dry. Note the condition of the wrack on the bench recording sheet.
    3) Avoid collecting flies, bugs and sand as much as possible
    4) Take photos of the wrack if possible. Record species of the wrack on Field recording sheet if you know the type of wrack (i.e., Macrocystis is large, brown, kelp).
    5) Place into zip lock bag, label and place in cooler on ice.

7. Collect coupon samples if applicable (see separate protocol).
8. Transport all samples to lab for processing within 2 hours of collection.
9. Make sure field sampling sheet is complete, including signatures for COC. Make copies for yourself and leave original copies with lab.

Laboratory Processing and Analysis

Laboratory Equipment and Supplies

Bench recording sheets
Membrane filtration apparatus and supplies
EPA Method 1600 supplies
IDEXX Enterolert apparatus and supplies
Ultra low freezer (-70 to -80 C)
Drying oven (to determine dry weights and moisture content of sand and kelp)
90 ml & 99 ml Di water (for dilutions if needed)
PBS (For coupons, use same PBS as for EPA Method 1600)
Graduated cylinders
Gloves
Timer
Weigh balance
Metal weigh boats
Plastic weigh boats
Disposable spatulas
100ml Disposable funnel for filtration for molecular analysis
Polycarbonate membranes filter, 47mm, 0.4um (HTTP0047, Millipore)
Pre-labeled 2 ml microtubes
Microtube rack
Freezer boxes for microtubes
Filter forceps
100% ethanol
Beakers
Alcohol lamp, tea candle or bunsen burner
Safety items: latex gloves, lab coat, safety glasses, freezer gloves

Additional items for coupon study

Bronson sonicator
2 L pp bottles
1 L pp bottles
1 L glass beaker

SOP for Filtration and Analysis of Water Samples

Procedures

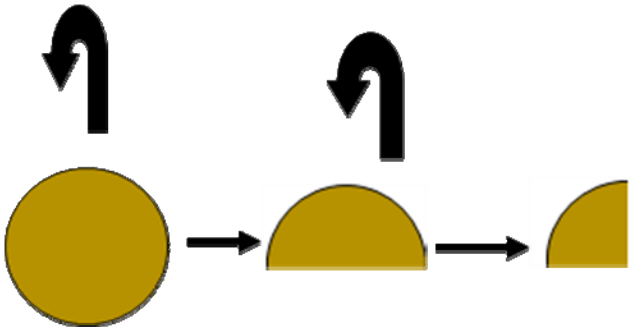
EPA 1600 analysis in *duplicate*
Please follow your standard protocol and dilution series.

Polycarbonate filtration for archiving filters for molecular analysis
The following steps describe filtration for polycarbonate filter only -- 100ml disposable funnel (individually wrapped) are used to avoid contamination

Remember to fill the "Filtration Bench Sheet" (i.e. Appendix B.1.)

1. Connect manifold, vacuum tubing, vacuum flasks etc.
2. Insert adaptor (supplied with funnels) into rubber stopper, place stopper on manifold, so it is ready to mount filtration funnels
3. One should test whether the vacuum assembly is working properly before moving forward.
4. Light the candle or alcohol lamp (for flame sterilization)
5. Wear gloves and use aseptic techniques from now on
6. Place corresponding 2ml microtubes in rack, unscrew cap to loose only (*ready for storing membrane filter after completion of filtration*)
7. Soak one pair of forceps in a beaker with 100% ethanol
8. Mount the filtration funnel (i.e. housing) on to the adaptor, Label the funnel with sample name.
9. Remove the funnel from the base and place it upside down on the bench on top of its lid.
10. Take one pair of forceps, flame sterilize, allow to cool a little, then pick up the grid filter from the filtration housing, discard the grid filter (*be careful not to damage the supporting filter underneath the grid filter*)
11. Use the same forceps to carefully pick up one polycarbonate membrane (*clear grayish membrane in-between paper separators, do not mistake the paper separator as the membrane filter*), and carefully place it onto the center of the supporting filter (*to replace the grid filter discarded in step 10*). Note: filter may be a bit hard to put on because of static charge. Open up just a little vacuum will help. In any case, always handle the membrane filter by the edge, and do not scratch the center of the filter.
12. Put the forceps back into the beaker with ethanol
13. Mount the filtration funnel (*that was set aside in step 9*) back onto the housing to secure the polycarbonate membrane (*make sure there is no gap between the edge of polycarbonate membrane and the bottom of the filtration funnel and that the edge of the membrane is not folded, i.e. no liquid should go through the housing/support without passing through the polycarbonate membrane first)*
14. Fill the funnel with 50ml water sample (NOTE: *depending on turbidity of the water, one may need to start with a smaller volume)*, open the vacuum valve and start filtering. This should filter relatively quickly <5min, add another 50ml to achieve total volume of 100ml. If the first 50ml takes more than 10min, do not add the additional 50ml, rather, filter the next 50ml with another filter. When two filters are used, the two filter can be placed into the same microtube (step 17).
15. Check off on the recording list, or record volume filtered (on each filter if necessary).
16. After all liquid passes through the membrane filter, rinse with just a little sterile PBS to wash off residual material from the wall of the funnel. When all liquid passes through, close vacuum valve and remove the funnel
17. Using two pairs of flame sterilized (*and cooled)* forceps, carefully fold the polycarbonate membrane filter twice on the housing. First, fold the membrane onto itself (about 1/2 of the diameter of the membrane) and hold in place with second forceps. Then, using the other pair of forceps alternately, gently fold membrane over again into a quarter, see diagram below). Place into the corresponding 2ml microtube (*check the label on the tube first*), screw the cap securely. *This is no need to fold it tight. It is actually preferred the*

filter was not squeezed tightly into the tube. Avoid touching the sample side of the membrane filter at all times.



18. Flash freeze the filter tube with liquid nitrogen or dry ice. Keep in liquid nitrogen or on dry ice till transfer to -80C freezer (organize the tubes in the freezer box first). If no liquid nitrogen or dry ice available, place the microtubes into -80C freezer ASAP.

SOP for Detecting and Enumerating Enterococci Bacteria in Beach Sand

Background
Sand and sediment in marine environments may serve as reservoirs of fecal indicator bacteria. The method described below was determined as part of the Bight '08 sand method evaluation study.

A video demonstrating this procedure is available on the SCCWRP website. The sand procedure and worksheets are also available for downloading at:
(http://www.sccwrp.org/ResearchAreas/BeachWaterQuality/BeachWaterQualityMonitoring/EvaluatingMethodsToMeasureFecalIndicatorBacteria.aspx)

Note: This method is recommended for extracting fecal indicator bacteria from coarse-grained sand. Sediment consisting of fines and clays may require alternative extraction methods such as sonication to remove attached bacteria.

Sample Collection (see Field Sample Collection)
Note: Sand samples will be collected using 50 cc conical tubes

Equipment and Supplies

Bench Recording and Calculation Sheet
Aluminum foil
Balance
Sterile 250 mL Nalgene wide-mouth bottles (2)
Sterile Phosphate-buffered Saline (PBS), EPA 1600 formulation
Sterile spatulas
Clean graduated cylinder
Multi-Timer
Drying Oven
Weigh Boats (acceptable for use in drying ovens at 103-105^{o}C)
All supplies and media for performing EPA Method 1600
Blood agar plates, TSA slants, sterile cotton-tip swabs, transfer loops (optional – for pigment testing)

Procedure for Extracting and Enumerating Enterococci from Sand

Part A: Enterococci in Eluant (i.e., "sandwater") from Beach Sand
Pour out sand from the 2 centrifuge tubes onto a clean foil, homogenize sample by mixing with a sterile spatula. (Don't toss these centrifuge tubes, save them for part C)
1. Place sterile 250mL Nalgene bottle on balance.
2. Tare balance and use a sterile spatula to transfer about 10g of homogenized sand into bottle. Record actual sand weight in Part A of the bench recording and calculation sheet.
3. Add 60mL sterile PBS to bottle; replace and tighten cap.
4. Shake bottle vigorously for 2 minutes.
5. Allow sand to settle for approximately 30 seconds.
6. Decant eluant into a second sterile bottle taking care to leave sand behind.
7. Add an additional 40mL sterile PBS to bottle. Swirl gently for 10 seconds.
8. Immediately decant this eluant as before into the second sterile bottle.
9. Perform EPA method 1600 on eluant using the eluant in place of an ambient water sample. The volumes for mEI should be adjusted to achieve 5 – 10 colonies/mEI plate without clogging the filter (recommend 0.01 ml, 1 ml, 10 ml). Eluants that are turbid may require further dilution.

(Note: Volumes filtered will vary based on bacterial concentrations and may not be the same for all samples.) You would only need <50ml of the eluant for EPA 1600.
10. With the leftover eluant, Filter at least one (preferably 2) polycarbonate filter (20ml/filter), using eluant in place of an ambient water sample. Please refer to above "filtration for molecular analysis".
11. Check off and record filtration work on the Filtration Bench Sheet (i.e, Appendix B.1)
13. Next day: count the mEI plates, and record results

13.1 pick isolates off one mEI plate, and conduct pigment testing (see below). If you are unable to perform the test. Save this plate in fridge and contact Donna.

13.2 save another mEI filter (>20 colonies only) in a 2ml microtube, freeze at -80C for molecular analysis. Check off and record CFU/filter on bench sheet.

Yellow pigmented *Enterococcus* isolates (optional).

Note: If you are unable to perform pigment test on isolates, please contact Donna. Yellow pigmented species (*E. casseliflavus*, *E. mundtii* and some *E. faecium*) are commonly associated with plants.

Pigment Testing Procedure

1. Sub-culture single colony (presumptive for enterococci) on blood agar plate (BAP) and incubate over night at 37°C. The BAP may be divided in half to streak 2 different isolates.
2. Make sure colonies on BAP are pure and typical for enterococci.
3. Swab a single colony and check for pigment by holding the swab under a lamp
4. A positive (+) result is YELLOW; the intensity may vary. If the pigment is light or weak, indicate the test as (+w). Cream colored colonies are NOT positive. If you are uncertain whether the pigment is weak vs cream, indicate (+/-).
5. Sub-culture 25% of colonies (at least 10 colonies per mEI plate) if possible; or if less than 10 colonies, sub all available colonies that appear typical for *Enterococcus*. Be sure to record total presumptive Ent count prior to selecting colonies.
6. For species identification (**optional**), perform speciation on 24h old culture from BAP; otherwise, subculture single colony to TSA slant, incubate at 37°C for 24h; check for growth/purity and refrigerate slant at 4°C until speciation can be performed.

Part B: Sand Dry Weight

Note: if you don't have a drying oven, sand can be stored frozen until later (see part C)

1. Tare balance and place weigh boat on scale. Record weight in Part B of bench recording and calculation sheet.
2. Add 5~10g of homogenized sand sample to weigh boat. Spread the material so the sand is not packed for easy drying. Record actual weight of boat plus sand in Part B of bench recording and calculation sheet.
3. Place weigh boat and sample in drying oven overnight at 103-105 °C.
4. Remove sample from oven and allow to cool until it can be handled safely (do not allow sample to sit out for >1 hour).
5. Reweigh boat plus sand; record dry weight in Part B of bench recording and calculation sheet.
6. Report CFU/g dry weight sand

Part C: Freeze sand for moisture content and/or molecular analysis

1. Place 5~10g of homogenized sand sample into one of the 50ml centrifuge tubes that you used in sampling, cap securely. Use kimwipe to wipe off sand particles on the outside the tube before capping, to avoid contamination. Place in ziplock bag labeled "moisture content analysis", store in -80C. (Skip step 1 if you have the capacity to do part B at your facility). One ziplock bag can be used for the whole Bight08 period.
2. Place rest of the homogenized sand into the other 50ml centrifuge tube, cap securely. Use kimwipe to wipe off sand particles on the outside the tube before capping, to avoid

SOP for Detecting and Enumerating Enterococci in Beach Seawrack/Kelp

Background

Seawrack, especially kelp washed on shore are known to harbor fecal indicator bacteria. Thus, wrack may lead to increases in fecal indicator bacteria in underlying sand and in beach water. The procedure for detecting and enumerating enterococci in wrack is based on Imamura et al., 2011 (in publication).

Equipment and Supplies

All equipment and supplies for EPA Method 1600, EPA 1604 and IDEXX Enterolert
Refer to "Field sampling equipment and Supplies"
Gloves
Large Ziplock bags
Balance
1L pp bottles
250ml pp bottle
PBS (EPA 1604 formulation w/ $MgCl_2$)
Large weigh boats that can withstand heating at 105°C
Drying oven
100% ethanol
Razors or scalpels (to cut wrack)

Sample Collection (See Field Sample Collection)

Procedure for Extracting Enterococci from Wrack

Part A: Enterococci in Eluant (i.e., "wrack water") from Wrack

1. Tare 1L bottle on balance.
2. Weigh out approx. 50 g of wrack into 1L bottle and record weight in Part A of bench recording and calculation sheet.
3. Wrack may be cut into smaller pieces if necessary using sterilized razor or scalpel
4. Add 500 ml PBS; replace and tighten cap
5. Shake vigorously for 3 minutes.
6. Allow contents to settle for 30 seconds. Gently pour off the eluant into the 250ml bottle, and this will be the "wrackwater" you use for EPA 1600 and filtration for molecular methods.
7. Quantitate enterococci in eluant using mEI agar. The volumes for mEI should be adjusted to achieve 5 – 10 colonies/mEI plate without clogging the filter. Eluants that are turbid may require further dilution.
8. Filter at least one (preferably 2) polycarbonate filter (50ml/filter), using eluant in place of an ambient water sample. Please refer to above "filtration for molecular analysis".
9. Check off and record filtration work on the Filtration Bench Sheet (i.e, Appendix B.1)
10. Optional: subculture presumptive enterococci isolates on mEI plate onto BAP for pigment testing (see part A).
11. Next day: place another mEI filter (>20 colonies only) in a 2ml microtube, freeze at -80C for molecular analysis. check off on bench sheet.

Part B: Determining Dry Weight

1. Tare balance and place weigh boat on scale. Record weight in Part B of bench recording and calculation sheet.
2. Add 10-15g of wrack sample to weigh boat. Record actual weight of boat plus wrack in Part B of bench recording and calculation sheet.
3. Place weigh boat and sample in drying oven overnight at 103-105 °C.

4. Remove sample from oven and allow to cool until it can be handled safely (do not allow sample to sit out for >1 hour).
5. Reweigh boat plus wrack; record dry weight in Part B of bench recording and calculation sheet.
6. Report CFU/g dry weight wrack.

Part C: Freeze unshaken wrack for molecular analysis (if leftover material available)

1. If extra wrack material is available (i.e., not all used in part A and B), place back in the original ziplock bag (get rid of air in the bag to reduce size). Store in -80C.

SOP for Using Concrete Coupons
to Assess Enterococcal Persistence and Growth

Background

In aquatic environments such as storm drains, *Enterococcus* bacteria may attach to surfaces and begin replicating or transition to a viable but non-culturable state by forming biofilm. Biofilm is a matrix formed when bacterial cells secrete substances that allow them to adhere to and grow on a surface. Once established, biofilms develop into a complex matrix containing diverse communities of bacteria. When biofilm containing enterococci forms on surfaces, bacteria may be sloughed off into the surrounding water by increased flow velocity, by scouring when debris move through the drain or due to natural aging of biofilm. Sloughed pieces of biofilm and bacteria may then be transported downstream where the bacteria colonize a new surface and continue their cycle of growth, or remain suspended in water discharged to beaches where they may impact water quality. This raises the possibility that bacterial growth related to urban runoff conveyance systems may be at least partly responsible for elevated concentrations of enterococci observed in urban runoff impacting beach water quality.

The goal of this study is to assess enterococci attachment and biofilm formation on concrete coupons as evidence of persistence and growth in urban runoff streams. The study will be conducted for at least a one month period during the dry season.

Study Sites

Coupons will be deployed at study sites selected by study participants. The sites will include storm water conveyance systems such as storm drains, concrete channels and creeks/streams. Study participants must conduct site surveys at least 2 weeks prior to deploying coupons to determine sampling logistics and feasibility based on the following criteria. The site must:

1. Have safe access
2. Include nuisance flows that reaches the beach or discharges directly into the ocean
3. Convey urban runoff flows that are not too high or too low. The water level must be high enough to cover the coupons, which are about 1" thick.
4. Include areas where coupons can be deployed such that they are not tampered with or lost during high flows. Need some kind of structure to tether coupons onto.
5. Have manhole access (for storm drains located underground)
6. Be located above the tidal prism
7. Be sampled by personnel that are certified to do sampling in confined spaces (depending on type of storm drain/channel).

Assessment of Enterococcal Biofilm Development

Concrete coupons measuring approx. 2" x 2" will be fabricated using concrete. The coupons will be primed in clean water for 2 weeks to leach out chemicals in concrete, wrapped in foil and autoclaved. The coupons will be need to be strung together using stainless steel wire and tethered and/or weighted down to prevent coupons from flowing downstream during high flows. They should be completely immersed in water.

Equipment and Supplies

See "Field sampling equipment and Supplies" page 3
Portable pH/conductivity/temp/DO meter
Turbidometer
Wire cutter (to cut coupons)
2L polypropylene (pp) bottles, sterile pre-filled with 500 ml PBS (for transporting coupons)
Items to measure flow rate & velocity
All equipment and supplies for EPA Method 1600 and IDEXX Enterolert
Sterile PBS (same formulation as for EPA Method 1600)
Sonicator

1L pp bottles, sterile
Ethanol 100%
Sterile DI water
Beakers, (1L) pyrex

Sample Collection

Overlying water

1. Prior to collecting coupons, collect a 125 ml water sample immediately upstream for enterococci enumeration.

Coupons

1. Remove aluminum foil from coupons and place in creek/storm drain. Coupons should be totally immersed underwater.
2. After a 1-week "incubation" period in storm water, remove two coupons (CC1 and CC2) for laboratory testing. Cut the last two coupons at the end of the wire using wire cutters. Avoid disturbing the remaining coupons.
3. Gently place each coupon into a separate 2L bottle pre-filled with 500 ml phosphate buffer saline (PBS) solution.
4. Place bottles upright in cooler filled with blue ice and transport to the laboratory within 2 hours of collection.
5. Remove two coupons weekly basis for a minimum of 4 weeks.

Characterization of Flow and Other Water Quality Parameters

Prior to removing the coupons, a water sample should be collected to temperature, conductivity (salinity), turbidity, pH and dissolved oxygen. Flow rate and velocity should also be measured.

Procedure for Extracting Enterococci from Concrete Coupons

Enterococci will be removed from each coupon using 3 different extraction methods resulting in 3 separate fractions (F) of cells:

- swirling the bottle containing the coupon to remove *loosely* attached cells (**F1**)
- shaking to remove *moderately* attached cells (**F2**)
- sonication to remove *firmly* attached cells (**F3**)

All three fractions of elutriates are processed similarly to water samples using mEI (Method 1600) and Enterolert.

1. Put on clean gloves
2. Label bottles: original 2L bottle = F1; clean 2L bottle with 500 ml PBS = F2; clean 1L bottle = F3.
3. Gently swirl original sample bottle containing coupon 3 times (F1). Eluant may be poured off into a clean 1L bottle (F1).
4. Change gloves, remove coupon and place into a clean 2L bottle containing 500ml PBS and cap; shake vigorously for 2 minutes. Save eluant for filtering (F2).
5. Remove coupon from bottle and place into a clean 1L pyrex beaker; add 500 ml PBS; sonicate coupon for 3 minutes at 30% output. Be sure to keep probe about 1 inch below water surface, changing the position so that the sound waves make contact with all sides of the coupon. Use clean 1L pyrex beaker for each coupon.
6. Pour off sonicated water into 1L pp bottle labeled "F3".
7. Rinse the probe with 100% EtOH between samples. Rinse off EtOH with sterile DI water or PBS.
8. Process eluants (F1, F2 and F3) using **Enterolert** and **EPA Method 1600**

Processing Eluants for Enterococci Enumeration using Enterolert and EPA Method 1600

Elutriates resulting from processing the coupons will be turbid and could clog filters during filtration. Thus, enterococci will be enumerated using Enterolert as well as mEI.

1. To obtain quantifiable results, start with using 10 ml volumes for Enterolert and 5 ml and 50 ml volumes for mEI. The volumes for mEI should be adjusted to achieve 5 – 10 colonies/mEI plate without clogging the filter.
2. For calculating concentrations of enterococci extracted, the concentrations from each fraction will be based on total counts in 500 ml PBS. The counts from F2 and F3 will be combined since they represent firmly attached cells. The results from both coupons (CC1 and CC2) will be averaged. The reported concentrations will be normalized to surface area of coupon (approx 24 inches total).

Yellow Colored Enterococci (optional)

Isolates from mEI may be tested for pigment and species identification. See Beach Sand SOP, part A.

***Enterococcus* Speciation (optional)**: contact Donna

Bench Recording Sheet and Chain of Custody

A COC/field sampling sheet will also be provided to sample collectors to record water quality parameters, flow rates and other field observations. A bench recording sheet will be provided to the lab to record enterococci densities from coupons. Please send electronic (or FAX) completed sheets (for COUPONS) to Donna every week. This is critical for determining whether adjustments should be made on testing volumes for the following week. Other worksheets should be sent to Meredith Raith at SCCWRP.

Compiled List of Equipment and Supplies

Field Equipment and Supplies

Chain of custody/field sampling sheet
Clip board, pens and sharpies
Orange vests
Boots/waders
Orange street cones (if needed for traffic safety)
1 L pp bottles for water
50 cc sterile centrifuge tubes (for sand: 2 for each sample)
Zip-lock bags (for sea wrack: 1 for each sample)
Paper towels
Gloves
Waste bag for gloves, paper towels, etc.
Coolers with blue ice packs
Hand sanitizer
First Aid Kit
Camera

Laboratory Equipment and Supplies

Bench recording sheets
Membrane filtration apparatus and supplies
EPA Method 1600 supplies
Ultra low freezer (-70 to -80 C)
Drying oven (to determine dry weights and moisture content of sand and kelp)
90 ml & 99 ml Di water (for dilutions if needed)
PBS (For coupons, use same PBS as for EPA Method 1600)
Pipets, Pipettors and Pipet Tips
Graduated cylinders
Gloves
Timer
2L pp bottles
1L pp bottles
250 mL Nalgene wide-mouth bottles
Weigh balance
Metal weigh boats
Plastic weigh boats
Disposable spatulas
100ml Disposable funnel for filtration for molecular analysis
Polycarbonate membranes filter, 47mm, 0.4um (HTTP0047, Millipore)
Pre-labeled 2 ml microtubes (labels will be provided by SCCWRP)
Microtube racks
50cc tube racks
Freezer boxes for microtubes (will be provided by SCCWRP)
Filter forceps
100% ethanol
Beakers
Alcohol lamp, tea candle or bunsen burner
Safety items: latex gloves, lab coat, safety glasses, freezer gloves
Razors or scalpels (to cut wrack)

Equipment and Supplies (cont)

Aluminum foil

Sterile spatulas
Clean graduated cylinder
Multi-Timer
Weigh Boats (acceptable for use in drying ovens at 103-105^{o}C)
Bench Recording and Calculation Sheet

Coupon Study: Additional Supplies

Field:

Portable pH/conductivity/temp/DO meter
Turbidometer
Wire cutter (to cut coupons)
PBS (phosphate buffered saline)
2L polypropylene (pp) bottles, sterile (pre-filled with 500 ml PBS)
Field equipment to measure flow rates & velocity

Lab:

IDEXX Enterolert
Bronson sonicator
2 L pp bottles
1 L pp bottles
1 L glass beaker

Additional Items for Pigment Testing

Blood agar plates
Sterile cotton-tip swabs
Transfer loops

Enterococcus speciation (optional): contact Donna