Lab Procedures

Collecting - Processing - Counting

STORMWATER MONITORING PROJECT

Collecting - For each outfall sample

1. Collect the sample in a 250 ml sterile bottle. Rinse the bottle and cap three times. Keep the fourth collection and cap tightly. Note: Fill to the shoulder of the bottle in order to leave enough room to allow sufficient mixing of the sample when processing in the lab.
2. Record the bottle ID on the Lab Datasheet (or at the bottom of the Field Datasheet).
3. Proceed to next outfall.

Processing Bacteria - For each water sample

1. Use distilled water for negative control (NC). It is not necessary to sterilize the distilled water.
2. Turn on incubator to 37°C (97°F).
3. Spray down lab work surface with isopropyl alcohol.
4. Washing and/or sanitize hands. Put on gloves if desired.
5. Label R cards (one per sample bottle, plus one NC) with:
   - Date
   - Outfall/Field Site ID
   - Sample Bottle Number
   - For negative control, write ‘NC’
   - For duplicate, write ‘dupe’ after outfall ID
6. Collect needed materials:
   - Micropipette
   - Sterile Micropipette tips
   - Forceps and sterilization materials if you choose to use them

Note: if you choose to use the forceps to lift the cover on the R card, make sure to sterilize before each use.

1. Sterilize the forceps.
2. Make sure the micropipette is set to 3 ml (3000 µL). This will look like 3.00 on the micropipette.
3. Shake the sample bottle 30 times ear to belt (moving the bottle in an arc from about your ear to your waist).
4. After shaking, take the sample as quickly as possible. Put a sterile tip on the micropipette. Press down on the top button until you hit the first stop. Submerge the micropipette tip in the sample and extract a 3 ml sample by releasing the top button while stirring and moving the micropipette up and down in the bottle.

Note: Release the top button slowly to prevent sucking up air bubbles in the tip. To find the first stop, press down on the top button gently until you feel a natural stopping point. You can push past this point if you exert more pressure but you should feel a defined stop.

IMPORTANT: Be careful not to lay the micropipette down flat or invert it as this will allow the sample in the tip to run back into the machinery of the micropipette.

5. Make sure the R card is on a level surface and carefully lift the top layer of the R card without touching the underside of the cover. Expel the sample water as droplets spreading it across the R card, but avoiding the very edges, by slowly pushing down on the top button of the micropipette. Go past the first stop on the micropipette all the way to the final stop to expel the sample. Gently lower the top cover of the R card. Do not press it down with your fingers.
6. Use button on the back of the micropipette to remove the tip into a ‘non-sterile’ container.
7. Wait 60 seconds to allow the R card coating to absorb the sample water. **Note**: You can also complete all of the samples first and then wait 60 seconds after the final sample before putting all of the samples in the incubator at the same time.
8. Place the R card into the incubator and record the time on the Lab Datasheet.
9. Incubate for approximately 23 hours.

Processing Turbidity - For each water sample

Step	Check for Accuracy:	To Calibrate:	To Use:
1	QUICK Press the POWER/CAL button (upper center button)	When 'STBY' is on screen, press and hold down the POWER/CAL button for 3 seconds. This will take you into calibration. You should see 'Cal 1' in the lower left corner of the screen	RINSE the vial 3 times with sterile water from a lab squeeze bottle.
2	Take 100 NTU vial from calibration kit	Start with 0 NTU calibration vial and invert slowly 3 times	INVERT 250 ml. lab-sample bottle 3 times and fill vial with sample water.
3	Slowly INVERT 3 times - careful to not introduce bubbles	Insert 0 NTU vial into AMTAST meter Match black bump with gray triangle	WIPE down outside of vial to remove fingerprints/water droplets.
4	WIPE down outside of vial to remove fingerprints	Press READ button and wait until '20' appears on screen	Insert vial making sure to match up the small dot on the lid with the arrow on meter.
5	Insert vial, making sure to match up black bump on lid with gray triangle	Replace 0 NTU with 20 NTU and press READ button; Cal symbol should blink Wait until '100' appears on screen	QUICK Press READ button. Value will appear in screen within 10-15 seconds
6	QUICK Press READ button	Replace 20 NTU vial with 100 NTU and press READ button Wait until '800' appears on screen	Record number on datasheet, remove and empty the vial.
7	If reading is between 97-103, continue to Column 4: 'To Use'	Replace 100 NTU vial with 800 NTU vial. Press READ button. Wait until STBY appears.	Repeat steps for all sample bottles.
8	If reading is outside range, continue to Column 3: 'To Calibrate'	Remove 800 NTU vial. Screen should read 'STBY', You are now ready to take a reading.	To turn off: QUICK Press POWER/CAL button.

Sterilization & Clean-up

1. Dispose of all extra sample water and then sterilize sample bottles and lids by putting a spritz of distilled water in each bottle. Microwave for 60 seconds at 100% power. If the bottles have been properly sterilized, you should see steam rising from them when you pull them out of the microwave. You may need to microwave longer depending on the power of your microwave.
2. Sterilize all used micropipette tips by putting a spritz of distilled water in the non-sterile container containing the used tips. Microwave for 60 seconds at 100% power. You may need to microwave longer depending on the power of your microwave. Sanitize your hands and then return the sterile tips to the tip container.
3. Put away all equipment.
4. Spray down workspace with isopropyl alcohol.
5. Wash and sanitize hands.

Counting, Documentation, Data Entry & Disposal - For each R card

1. Count the number of large, dark blue/green dots on the R card. Ignore any pinhole-sized or very faint dots.
2. Record the pertinent information on the Lab Datasheet.
3. Remember to also calculate the total number of CFU/100 ml.
4. Calculating for 3 ml samples: # of green dots on card X 33.3. Round to the nearest whole CFU.
5. Place R cards in a plastic baggie, squirt about 1 ml of isopropyl alcohol into the bag and seal. Swirl the liquid around the baggie to ensure all the R cards are covered and then dispose of the plastic baggie in the trash.
6. Take a photo of the Lab Datasheet and the R cards and send to: surveydatasheets@googlegroups.com and woodc@umich.edu
7. Open the data entry form at **https://friendsofsahlissea.org/volunteer-resources/**. Choose 'sign in as Guest' to open the form and complete the form with the data from your survey. Be sure to check the 'complete' box in the upper left corner of the screen and log out when complete.

Counting - Estimation Method

If there are a lot of dots, you can use the Estimation Method developed by the Whatcom Conservation District:

1. Only use this method if there appears to be >60 colonies on the R card.
2. Choose 5 squares randomly (these squares must be populated, no blank squares), and count them. Record the number of colonies for each square.
3. Take the average of the 5 squares and multiply by how many total squares are populated on the R card.
4. While counting all the populated squares on the card, you can combine squares, if needed. (Ex. Combining 2 squares that are only half-populated = 1 square)
5. Most labs look for an R card or plate count of 20-60 cfu/100 ml