

Bacteria Procedures

Collecting – Processing – Counting

COLLECTING For Each Outfall

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 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 Bacteria worksheet.
3. Proceed to next outfall
2. Make sure micropipette is set to 3 ml (3000 μ L). 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.

PROCESSING For each water sample

1. Use distilled water for negative control. 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. Wash and/or sanitize hands. Put on gloves if desired.
 5. Label R cards (one per sample) with:
 - Date
 - Outfall/Field Site ID
 - Sample Bottle Number
 - For negative control, write “NC”
 - For duplicate, write “dup” after the outfall ID
 6. Collect needed materials:
 - Micropipette
 - Micropipette tips
 - Forceps and sterilization material if you choose to use them
- For each water sample:
- Note:** Release the top button slowly to prevent sucking up air bubbles into 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. (Note: There is a helpful video linked under the training tutorials tab on the website)
- 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 on 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.

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.

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 worksheet
9. Incubate for approximately 24 hours
10. Note: For NC use sterile distilled water.

STERILIZATION AND CLEAN-UP

1. Dispose of all extra sample water and then sterilize sample bottles by putting a spritz of distilled water in each bottle and then microwaving 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 little distilled water in the unsterile container containing the used tips and then 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

For each R-Card:

1. Count the number of green dots on the R-Card
2. Record the pertinent information on the bacteria worksheet
3. Remember to also calculate the total number of CFU/100 ml.

4. Calculation for 3ml sample: # of green dots on card x 33.3. Round to the nearest whole CFU.

DOCUMENTATION AND DISPOSAL

1. Take a photo of the bacteria worksheet and the R-Cards and send to **woodc@umich.edu**
2. Place R-Cards in a plastic baggie, squirt about 1 ml of isopropyl alcohol in, and seal the baggie. Swirl the liquid around the baggie to ensure all the R-Cards are covered and then dispose of the plastic baggie in the trash.