

Standard Operating Procedure for Detecting and Enumerating Enterococci Bacteria in Beach Sand

Supply List

Balance
Sterile 250 mL Nalgene wide-mouth bottles (2)
Sterile Phosphate-buffered Saline (PBS)
Sterile spatulas
Clean graduated cylinder
Multi-Timer
Drying Oven
Weigh Boats (acceptable for use in drying ovens at 103-105°C)
All supplies and media for performing [EPA Method 1600](#)
Bench Recording and Calculation Sheet

Procedure

Part A: Bacteria in Eluant from Beach Sand

Perform in triplicate for each of the 3 sand samples.

1. Place sterile 250mL Nalgene bottle on balance.
2. Tare balance and use a sterile spatula to transfer about 10g of 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. (Note: Volumes filtered will vary based on bacterial concentrations and may not be the same for all samples.)

Part B: Sand 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 sand sample to weigh boat. 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.