

## **Environmental Health and Environmental Justice Audubon/AMBIENT 2004**

### **Laboratory Exercise for Coliform Analysis**

Purpose of Measurements: The safety of recreational waters (e.g. beaches and swimming pools) is evaluated by measuring the presence of “indicator” microbes in the water. When water is contaminated by sewage it is not safe for swimming because sewage contains micro-organisms that cause disease (pathogens). In other words if you swim in water that has been contaminated by sewage you increase your likelihood of getting sick because of possible contact with pathogens.

“Indicator” microbes are natural inhabitants of the gastro-intestinal tract of humans. If a water body has been contaminated with human waste (e.g. sewage) then indicator microbes are present in the water. Indicator microbes do not generally cause disease. The presence of indicator microbes “indicates” the presence of sewage and therefore the presence of disease causing organisms.

Indicator microbes that are measured to determine if a beach is safe for swimming include: total coliform, fecal coliform, E. coli, and enterococci. In this laboratory exercise we will be measuring total coliform in water and sediment along the beach. If total coliform concentrations exceed 1000 organisms per 100 milliliters on average, then the beaches will be declared un-safe for swimming. We are including sediment analysis because researchers believe that sediments can represent a significant source of indicator microbes in addition to sewage sources.

Principle of Analysis: The liquid contained in the test tubes used for analysis contain nutrients necessary for total coliform to grow plus bromocresol purple, a pH indicator. When coliform are present in a sample they will grow in the test tube and cause a change in the pH resulting in a change in the color of the pH indicator to either yellow or green. The method utilized in this experiment is known as a “presence – absence” test. It will indicate the presence or absence of total coliform in your sample. It will not provide the concentration of the indicator microbe in the water sample.

# **PROCEDURES for the Coliform Test Kit**

## **SAMPLE COLLECTION**

- 1) Make sure your gloves are on.
- 2) Label your Whirlpak bags using a waterproof pen. You will be collecting 4 samples: one water sample from Hobie Beach, one soil sample from Hobie Beach, one water sample from Virginia Beach, and one soil sample from Virginia Beach.
- 3) Enter the sample identification number and date/time of sample collection in your data collection sheet. Note location of sample collection.
- 4) Collect your water and soil samples using the pre-sterilized Whirlpak bags provided. One bag for the soil and another bag for the water. For soil sample collection use a sterile spoon to collect the soil sample. A couple of scoops of soil will be fine. For the water sample collection submerge the bag under water and be careful not to stir the bottom sediments. 50 ml of water is sufficient. \*Note: Do not touch the *inside* of the Whirlpak bag to avoid contamination from your hands/gloves.
- 5) Seal the whirlpak by folding the top portion several times. You may need to expel some of the air from the bag to do this.
- 6) Place on ice until reaching your lab.

## **SAMPLE ANALYSIS**

- 1) Label the tops and glass portion of your tubes.
- 2) Carefully remove the cap of lactose broth tube (tube containing the purple liquid). For the water sample add 1 mL water sample using the pre-sterilized dropper provided. Use the small pre-sterilized metal scooper to add the soil to a separate tube.
- 3) Keep tubes in upright position at room temperature for 24 hours.
- 4) Observe the color of the liquid in the tubes after 24 hours. If the liquid is still purple, coliforms are not present and results are therefore negative. If after 24 hours the broth is green or yellow, then coliforms are present and this would indicate that coliform were present in your sample. The green/yellow liquid indicates a positive result.

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**Microbe Data Collection and Analysis Work Sheet**

Group Members: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Sample Collection Table

Sample ID	Date Collected	Time Collected	Sample Type (soil or water)	Sample Location (e.g. swash zone, knee deep water, Hobie vs. Virginia Beach, etc..)	Environmental Conditions During Sampling (e.g. sunny, windy, etc..)

Notes: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Analysis Sheet

Sample ID	Date Processed	Time Processed	Date Observed	Time Observed	Result: Positive (yellow) or Negative (purple)

Notes: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
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