Bacterial Plating Instructions

Adapted from workshops conducted by Dr. Susan Laramore, FAU Harbor Branch
Also view video at http://shellfish.ifas.ufl.edu/

Collecting Samples

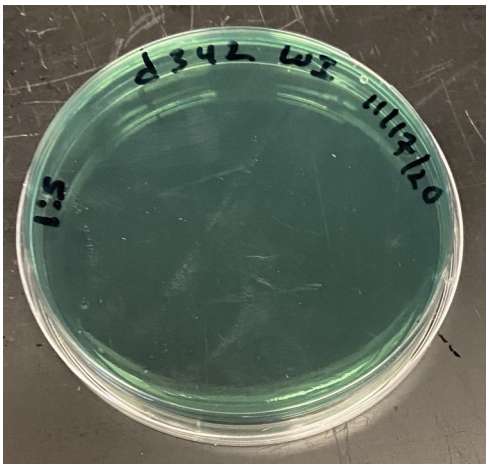
Components

- Whirl-pack bags (sterile)

Instructions

1. Collect water samples from problem areas (“hot spots”) in hatchery, such as incoming water, larval tank water, maturation tank water, larvae, algae stock cultures, and air supply.
2. Fill whirl bag with 200 ml water sample from each area in the hatchery and seal.
3. Label bag with YYYY.MM.DD - Business Code - Sample Location. Keep log sheet with description of each sample.
4. Plate and ship samples on the same day they are collected.

Plating Samples



Components

- Agar Plates
- 1000 µl Pipette tips
- 100-1000 µl Pipettor
- Spreader
- 10 µl loop
- Parafilm

Instructions

1. Label the bottom of the agar plate with the dilution, sample location, and date.
2. Place a sterile pipette tip on the pipettor. The same pipette tip can be used for multiple dilutions of the same sample. However, discard pipette tip and use a new sterile tip for samples taken at different locations.
3. Use a dilution ratio of 1:5 and 1:10 for samples taken from algae tanks, incoming tank water, and well water samples. Use a dilution ratio of 1:5, 1:10, and 1:100 for samples taken from tank water where animals such as larvae and post-set are present.

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Bacterial Plating Instructions (continued)

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Plating Samples (continued)

Components

4. Set the volume on the pipette. This will depend on the dilution ratio being performed

    - 1:5 - Set pipettor to 200 µl
    - 1:10 - Set pipettor to 100 µl
    - 1:100 - Use a 10 µl loop

To determine presence or absence (or for surfaces)

- Take a swab and streak on the plate in a zigzag fashion
- To save on plates use a marker and draw a line down the middle of the plate (media side) and streak on either side. This allows you to use the plates for 2 or more samples.

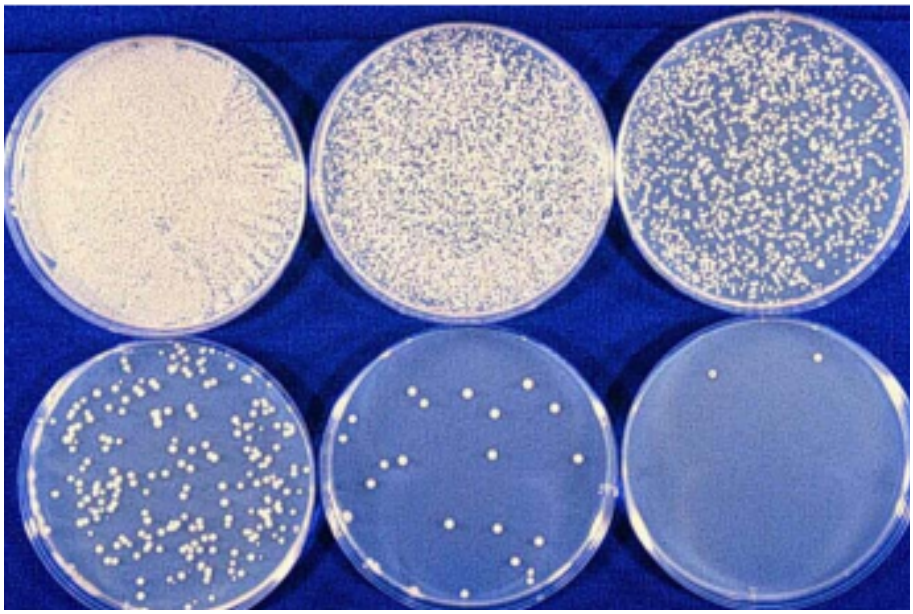
Quantification of Bacterial Load using 10 µl loop

- Label and date the bottom of the agar plate
- Use a premeasure disposable 10 µl loop (1/100 of a ml) and spread over entire plate
- Incubate plate upside down in a cool, dry place like a plastic container with lid
- Remove from incubator (or tub) after 24 hours and count colonies

Bacteriology Plating Interpretations

Counting Colonies

- Multiply by dilution factor used
- Example: 10 µl (0.01mls; dilution factor = 100) were spread on the plate and 20 colonies counted.
  - Total colonies = 100 x 20 or 2000 CFU/ml
  - CFU = colony forming units



Multiple types of colonies

- Count colonies with different colors, shapes, etc.
- Multiply by dilution factor and then add total
- Example:
  - 15 yellow and 36 green Vibrio colonies from a 10 µl sample
  - 100 x 15 yellow = 1500 yellow Vibrio CFU/ml
  - 100 x 36 green = 3600 green Vibrio CFU/ml
  - 1500 yellow + 3600 green = 5100 total Vibrio CFU/ml

Bacterial Plating Interpretations (continued)

Interpretation

- Generally, green colonies are pathogens and yellows colonies are non-pathogenic.

How much is too much?

- Algae (starter cultures) - no Vibrio!, likely some bacteria on marine agar (<10 CFU/mL)
- Tanks - some Vibrio
  - Total bacteria: up to 5,000 CFU/ml
  - Vibrio bacteria: <1,000 CFU/mL (no greens)