

BACTERIAL PLATING INSTRUCTIONS AND INTERPRETATION

SHELLFISH BACTERIAL WORKSHOP

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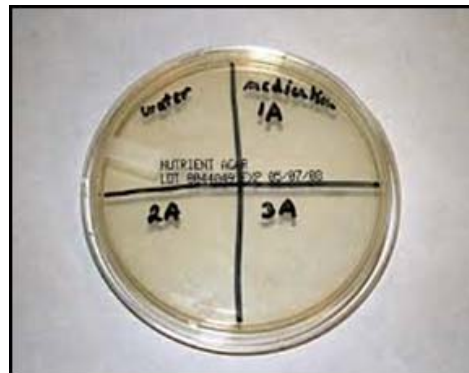
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Spread Plate Method

- This is the easiest and most common plating method
- Use pre-prepared plates or make and pour your own
 - Add 12 mls of sterile media into a petri dish and let solidify – few hours
- A small volume of water is either added to the surface of the plate and spread or is streaked on the plate
- The surface of the plate can not be too moist or the water will not sink into the media making colonies difficult to count (i.e. spreaders)
- Seal plate and incubate (tub with a lid if no incubator available)
- Count colonies, make necessary calculations due to dilutions, and interpret results

Presence or Absence of Bacteria

- To determine presence or absence (or for surfaces)
 - Take a swab and streak on the plate in a zigzag fashion
 - To save on plates using a marker, 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
 - “peace” sign (3 samples) or in quarters (4 samples)

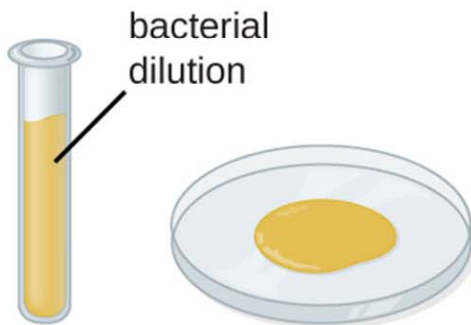


Quantification of Bacterial Load

- Add 0.1 ml (1/10 of a ml) or 0.25 (1/4 of a ml) ml to the plate and spread over entire plate with a spreader (hockey stick)
- Or use a pre-measure disposable 10 μ l loop (1/100 of a ml) and spread over entire plate
- Remove from incubator (or tub) after 24 hours and count colonies

Spread Plate Method

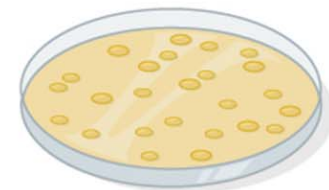
1 Sample (0.1 mL) poured onto solid medium



2 Spread sample evenly over the surface

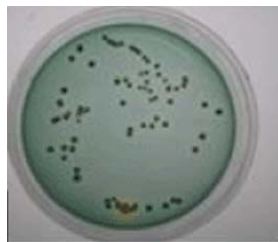


3 Plate incubated until bacterial colonies grow on the surface of the medium



Counting Colonies

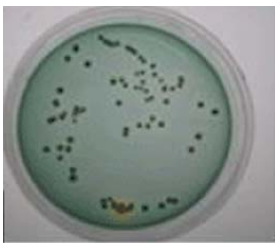
- Multiply by dilution factor used
- Using the examples above:
 - A) 0.1 mls were spread on the plate and 20 colonies counted. Total colonies = 10×20 or 200 colony forming units (CFU)/ml
 - B) 0.25 mls were spread on the plate and 20 colonies counted. Total colonies = 4×20 or 80 CFUs/ml
 - C) 10 μ l (0.01mls) were spread on the plate and 20 colonies counted. Total colonies = 100×20 or 2000 CFU/ml



Counting Colonies

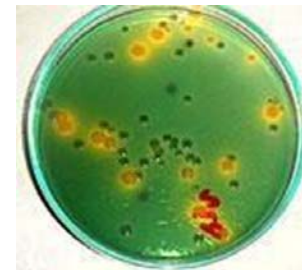
Colonies of same type

- Multiply by dilution factor used
- Using examples above:
 - A) 0.1 mls were spread on the plate and 20 colonies counted. Total colonies = 10×20 or 200 colony forming units (CFU)/ml
 - B) 0.25 mls were spread on the plate and 20 colonies counted. Total colonies = 4×20 or 80 CFUs/ml
 - C) 10 μ l (0.01mls) were spread on the plate and 20 colonies counted. Total colonies = 100×20 or 2000 CFU/ml



Multiple types of colonies

- Count colonies with different colors, shapes, etc.
- Multiply by dilution factor
- Add total
- Example:
 - 15 yellow and 36 green Vibrio colonies from a 0.1 ml sample
 - $10 \times 15 = 150$ yellow Vibrio colonies per ml
 - $10 \times 36 = 360$ green Vibrio colonies per ml
 - $150 + 360 = 510$ total Vibrio colonies per ml

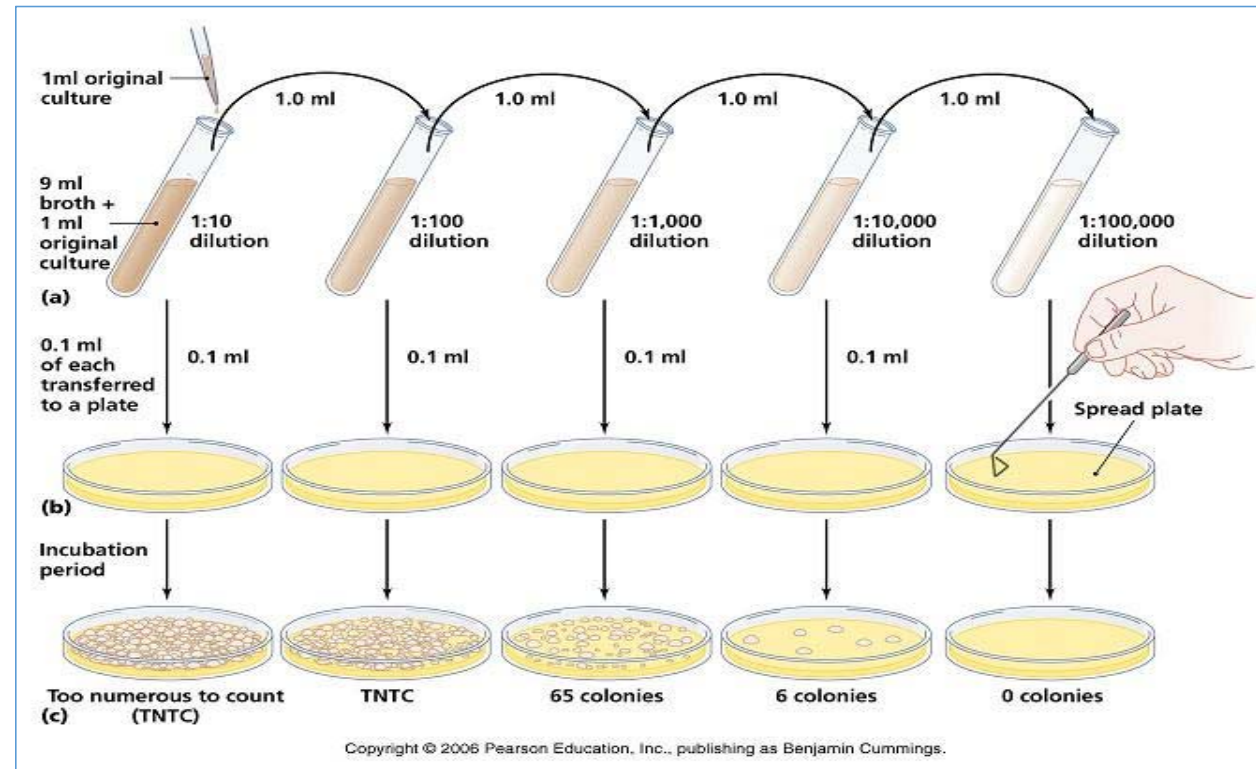


Multiple types of Colonies

- First count all colonies for total count of bacteria (marine agar) or a total count of Vibrios (TCBS) and multiply by dilution factor
- Then count colonies with different colors, shapes, etc. and do repeat the above
- Example:
- 15 yellow and 36 green Vibrio colonies from a 0.1 ml sample
 - $10 \times 15 = 150$ yellow Vibrio colonies per ml
 - $10 \times 36 = 360$ green Vibrio colonies per ml
 - $150 + 360 = 510$ total Vibrio colonies per ml

Serial Dilutions

- Typically not needed with Vibrio selective (TCBS) plates
 - Remember you are already typically diluting 1:10 with spreader plates
 - At most 1 1:10 dilution will be needed
- Often needed for total counts (Marine agar plates)
 - 1:10 dilution will be needed
 - 1:100 or 1:1000 dilution may be needed



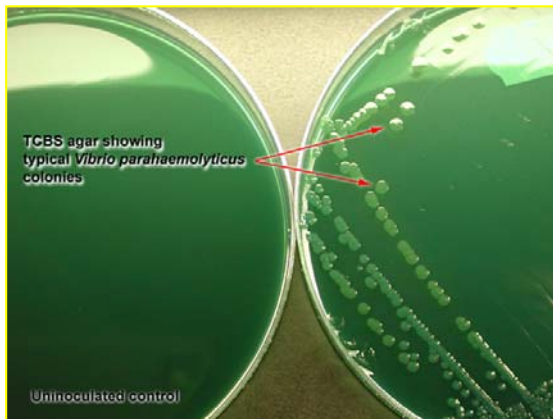
Serial Dilutions

- The typical range of colonies preferred for counts is 30-300.
- Less than 20 may not give you an accurate count
 - if samples have been diluted (too dilute)
- More than 200-300 is considered TNTC and should typically be diluted to give a more accurate count

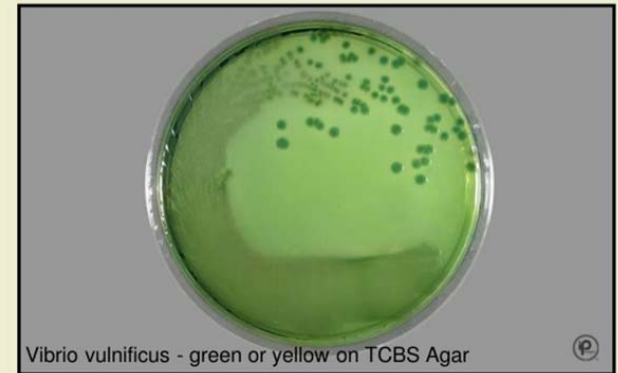


Interpretation

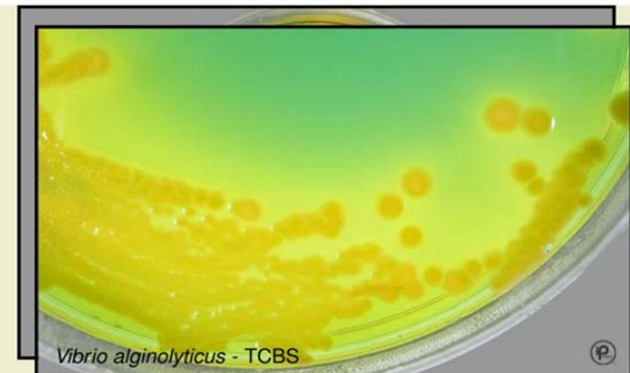
- Type
 - Pathogenic or non-pathogenic
 - TCBS – easy
 - Greens are pathogens & yellows are generally not
 - You do not want to see greens!!

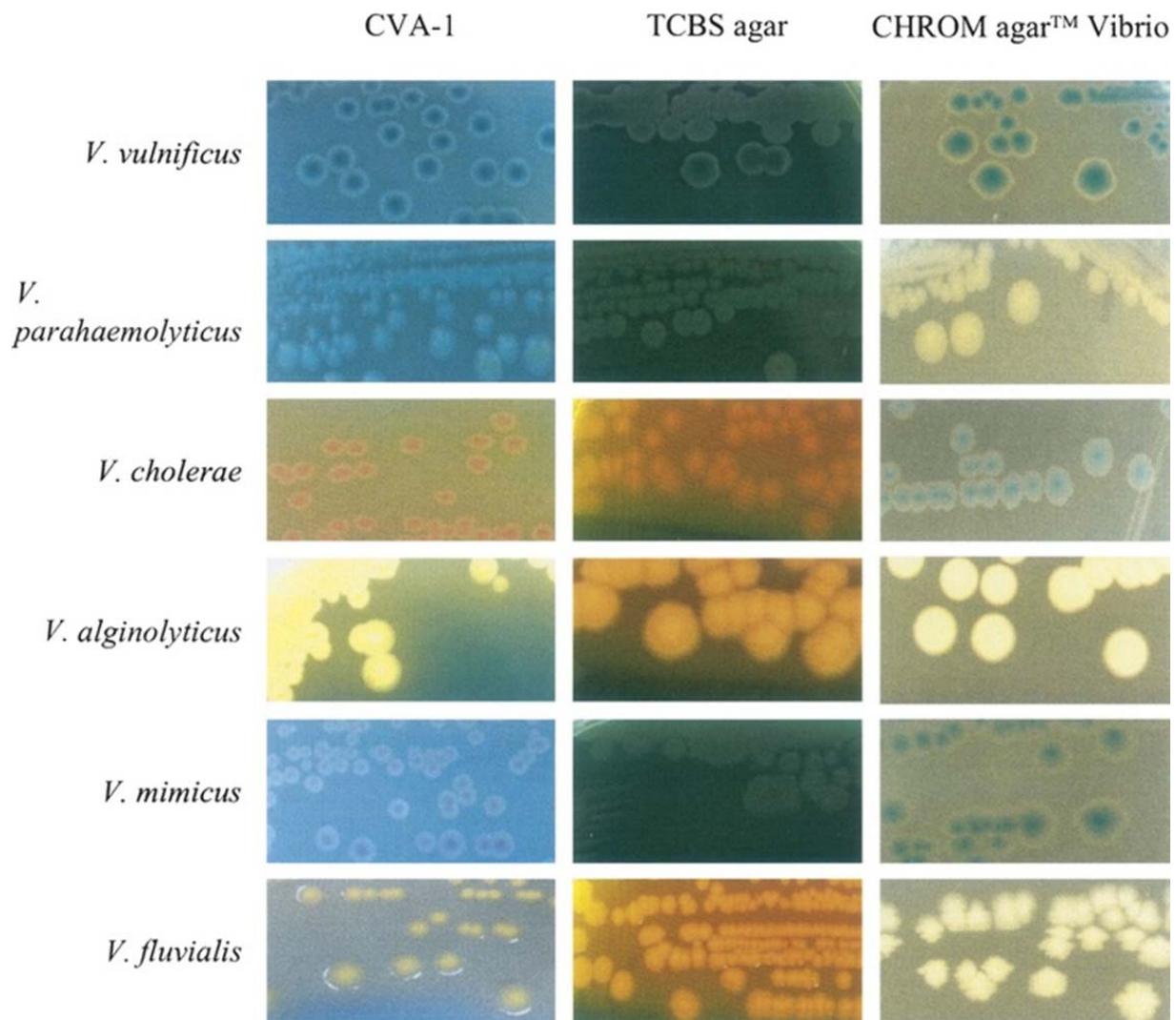


COLONY MORPHOLOGY



COLONY MORPHOLOGY





Interpretation, Guidelines and Recommendations

- Number
 - How much is too much?
- Depends on source and pathogenicity
 - Algae (starter cultures) – no Vibrio!, likely some bacteria on marine agar (<10 CFU/ml)
 - You will see some increase as you go up to carboys and higher but should not jump substantially
 - Tanks – some Vibrio, more total counts
 - Depend on water source - whether RAS, flow-through, size of bivalves
 - Larger size = more bacteria
 - Total – up to 5,000 CFU/ml (I like to see no more than 1,000)
 - Vibrio - < 1,000 CFU/ml (No greens!) (I like to see no more than 100)
- Recommendation
 - Determine what counts are in each system and in algae at start up and when there are no problems
 - Periodic bacterial monitoring of systems will give you an idea of background in your facility that does not cause a health issue