

The left side of the slide features a decorative design consisting of several vertical stripes in shades of light blue and teal, and a cluster of five teal circles of varying sizes arranged in a roughly circular pattern.

HOW TO MONITOR FOR BACTERIA

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WHY MONITOR FOR FECAL BACTERIA

- Fecal bacteria is one of the largest impairment sources of streams
 - For example in Virginia; >9,154 miles
- Waterbodies with fecal bacteria often have other issues such as increased sediment and nutrients
- High levels of fecal bacteria in waterbodies increase the risk of illness



Now on YouTube! Search 'Attack of the E. coli' or go to www.youtube.com/watch?v=fyY6YF9xtzc



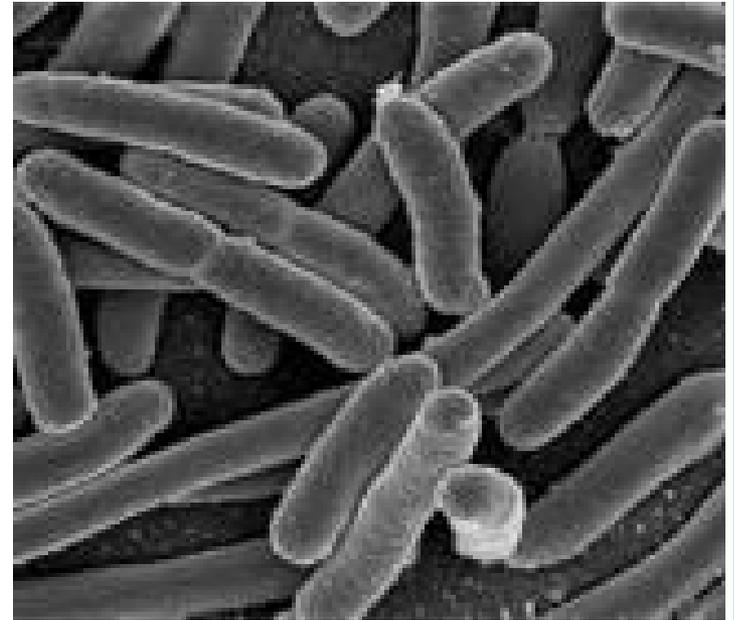
THE GREAT DEBATE- CFU vs. MPN

- Culture tests report results in CFU or MPN
- CFU or Colony Forming Units is the number of bacteria colonies that formed from the original bacteria in the sample
- MPN or Most Probable Number is a statistical estimate of the number of bacteria that were present in the sample
- There are some minor differences in the two methods but results are essentially the same
- Some states only accept data reported as CFU or MPN
 - A few states get around this by reporting as bacteria per 100 ml



ESCHERICHIA COLI (E. COLI)

- Gram negative, rod shaped, facultative anaerobic bacteria
- Normally found in the digestive tracks of warm blooded animals
- Usually monitored in freshwater
 - Single samples with >235 *E. coli* or a geometric mean of five samples >126 per 100 ml sample indicates excessive bacteria levels



FECAL COLIFORM

- Typically found in the digestive tract of warm blooded animals
 - *E. coli* and other species are members
- Most states have moved to sampling for *E. coli* or Enterococcus
- Fecal coliform monitoring still required in shellfish waters
 - Average levels must be <14 and single maximum readings no more than 49 MPN or 31 CFU



ENTEROCOCCUS

- Gram positive, spherical shaped, facultative anaerobic bacteria
- Normally found in the digestive tracks of warm blooded animals
- Usually monitored for in saltwater (beaches)
- Excessive bacteria levels typically indicated by:
 - Salt water samples with $>10^4$ enterococci or an average >35 per 100 ml sample
 - Freshwater samples with >61 enterococci or an average >33 per 100 ml sample



POPULAR METHODS TO MONITOR FOR FECAL BACTERIA

- Presence/Absence
- Coliscan Easygel
- 3M Petrifilm
- LaMotte BioPaddle
- Colilert/Enterolert
- Membrane Filtration
- Multiple Tube Method



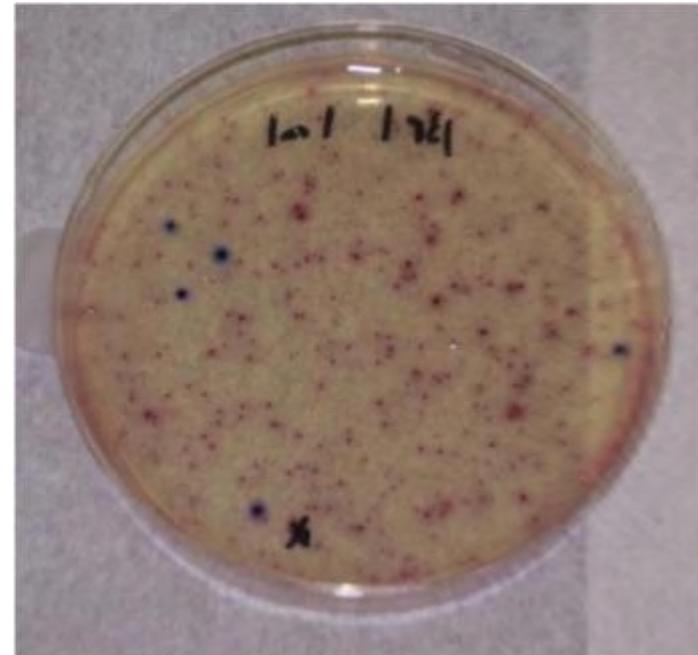
PRESENCE/ABSENCE

- Simplest culturing method
- Sample placed in a bottle containing nutrient media
- Color change/glow under UV or similar reaction indicates presence of the bacteria
- Results in 24 hours
- Normally used to test drinking water quality as no fecal bacteria should be present
- Can be used for education/awareness



COLISCAN EASYGEL

- Does not require a laboratory
- Tests 1 to 5 ml of sample
- Results in 24 to 48 hours
- *E. coli* appear dark blue to purple
- Results are in CFU
- Good to locate bacteria ‘hot spots’ and track overall trends



1 ml sample with 5 *E. coli* colonies



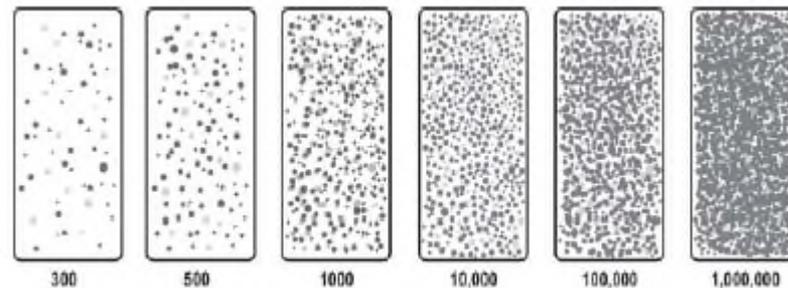
3M PETRIFILM

- Non-laboratory based test to detect *E. coli* and other coliform bacteria
- Tests 1 ml of sample but can be coupled with membrane filtration to test 100 ml
- *E. coli* appear dark blue with gas bubbles
- Results are in CFU
- Good for screening and education/awareness



LAMOTTE BIO PADDLES

- Non-laboratory based test to detect
 - Total coliform
 - Fecal coliform
 - *E. coli*
- Can be used to Calculate Total Viable Count and Total Colony Count from a 40 ml sample volume (for counts <300)
- Estimate colony density >300
- CFU results
- Good for screening and education/awareness

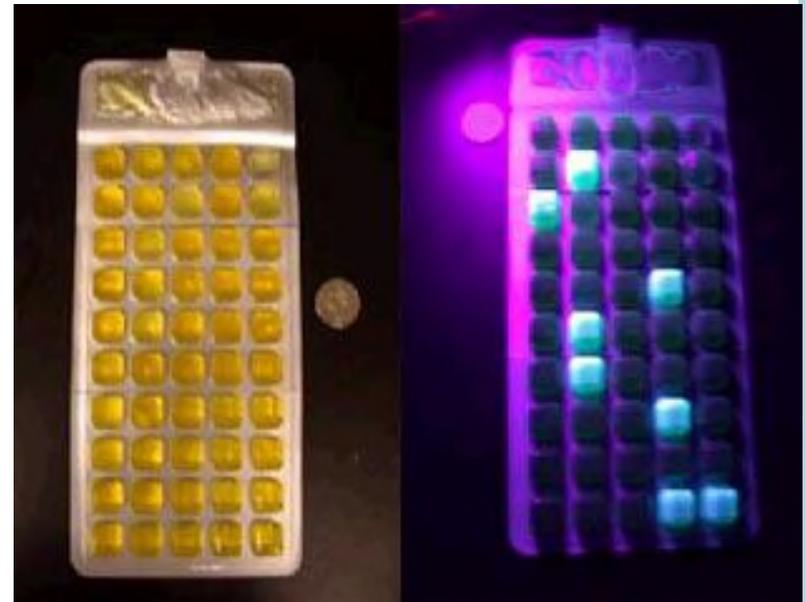


Approximate Colony Density (CFU/100mL)



COLILERT/ENTEROLERT

- Most popular lab-based method due to relative low cost and ease of use
- Results in 18 to 24 hours
- Colilert
 - Total coliform wells turn yellow
 - *E. coli* wells are yellow AND glow under a UV
- Enterolert - Enterococcus positive wells glow under a UV light but no color change
- Results in MPN
- Test is susceptible to optical brightener/pesticide interference



MEMBRANE FILTRATION

- Filter sample through a fine pore (< 0.6 μm) filter
- Tests up to 100 ml of sample
- Results in 18 to 24 hours
- Colony color depends on nutrient media
- Results reported as CFU
- Requires autoclave and vacuum pump and related laboratory equipment



MULTIPLE TUBE METHOD

- Not used as much since introduction of Colilert/Enterolert
- Multiple test tubes of media/auger inoculated with several sample volumes
- Results in 3 to 5 days
- Number of test tubes with a positive reaction determines the MPN result



Test Method	Presence /Absence	Coliscan Easygel	3M Petrifilm	LaMotte Bio-Paddle	Colilert/ Enterolert	Membrane Filtration	Multiple Tube
Lab Required	No	No	No	No	Yes	Yes	Yes
Min Detection Limit	0	<20	<100	<40	<1	<1	<1
Max Detection Limit	1	>6000	>6000	>1,000,000	>10,000	>10,000	>10,000
Unit of Measurement	N/A	CFU	CFU	CFU	MPN	CFU	MPN
Consumable Cost	\$6.50	\$2.50	\$3.00	\$6.00	\$6.50	\$2.50	\$2.00
Equipment Cost	\$0	\$100	\$100	\$100	\$15,000	\$25,000	\$25,000
Time Spent Per Sample	1 min	1 min	1 min	< 1 min	5 min	>10 min	>10 min
Incubation Time (Hrs)	24	24 to 48	24	18 to 24	18 to 24	18 to 24	72 to 120
EPA Approved	Yes	No	No	No	Yes	Yes	Yes

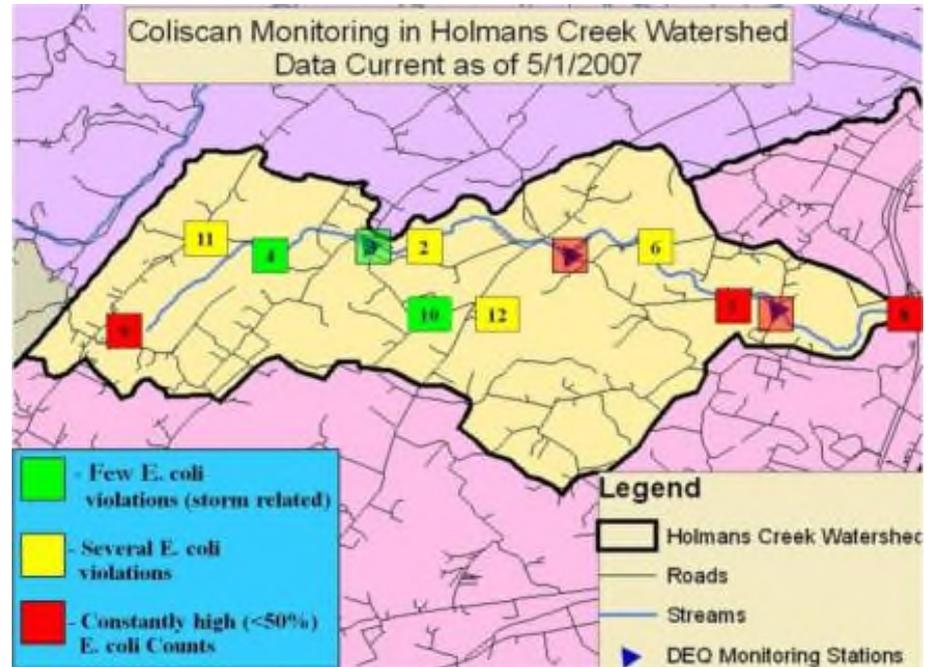
EFFECTIVE MONITORING STRATEGIES

- “Shotgun” method
- Targeted source identification/verification
- Carpet sampling (“B-52” method)
- Routine sampling to assess baseline conditions
- Storm event sampling



SHOTGUN METHOD

- Sample stations randomly scattered in the watershed
 - Sampling from public access points like bridges
- Ideal for initial recon to find bacteria “hotspot” segments
- Moderate labor and cost intensive



TARGETED SAMPLING

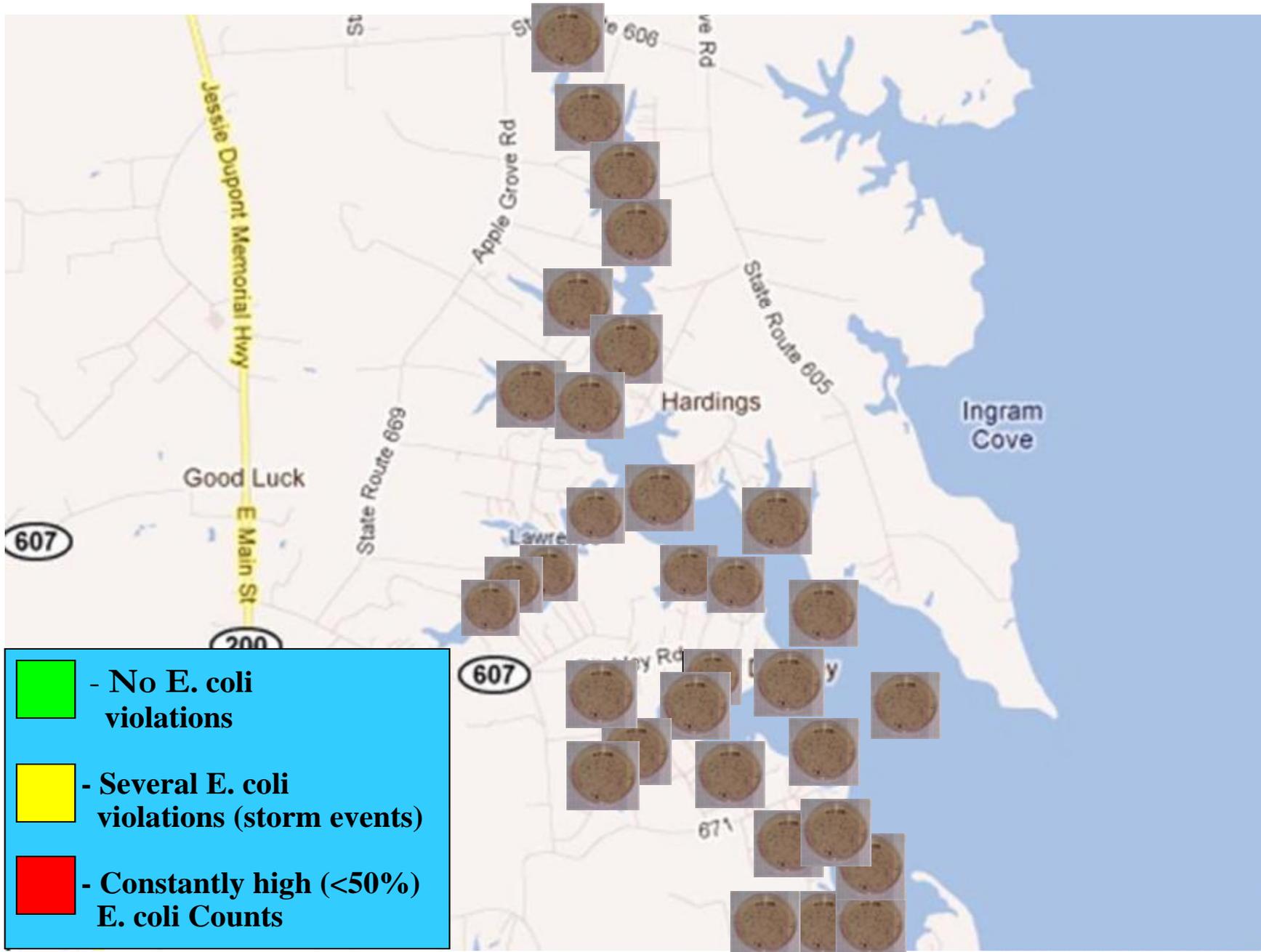
- Sample likely sources of fecal bacteria
 - Sewer line crossings or near septic systems
 - Areas of known dense wildlife or animal activity
- Good to quickly rule out or confirm fecal sources
- Requires planning to identify sources and may require landowner access



CARPET SAMPLING

- Sampling every possible source and waterbody segment at one time
- Extremely labor and cost intensive
- Provides the most detailed picture of fecal sources in the shortest amount of time





ROUTINE MONITORING

- Sampling part of overall monitoring strategy
- Can be used to assess baseline conditions, or target recreational waters not otherwise monitored
- Minimal additional effort required, but sample holding times can become a concern

Surfrider Sites - Blue Water Task Force Monitoring

RIDEM Primary Contact Recreational/Swimming Geometric Mean Density - Not to exceed 35 enterococci per 100 mL.

RIHealth standards at licenced beaches: Not to exceed 104 enterococci per 100 mL

Monitoring Location	1/20	* 2/24	3/17	4/21	5/18	6/29	7/27	8/24	9/28	10/26	11/23	12/21	GeoMean
	----- Most Probable Number of Enterococci per 100 mL -----												
Watch Hill Harbor	<10	10	10	<10	<10	42	20	<10	<10	<10	42	10	<10
Watch Hill	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Misquamacut DEM Surf Area	<10	<10	<10	<10	<10	10	<10	<10	<10	<10	<10	<10	<10
Fenway Beach	<10	<10	<10	<10	<10	31	<10	10	<10	<10	64	<10	<10
Deep Hole	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
The K's	<10	<10	<10	<10	<10	10	20	10	<10	<10	<10	<10	<10
Conant Ave.	<10	<10	<10	<10	<10	42	64	42	<10	<10	<10	10	<10
Scarborough Beach South	<10	10	42	<10	<10	137	<10	<10	<10	<10	<10	<10	<10
Scarborough DEM Surf Area	<10	<10	<10	<10	<10	42	31	<10	<10	<10	<10	<10	<10
Monahan's Dock	<10	-	<10	<10	<10	178	10	<10	<10	<10	<10	<10	<10
Narragansett Pier Beach Steps	<10	<10	<10	<10	<10	53	<10	<10	<10	10	<10	<10	<10
Bonnet Point	10	<10	<10	<10	<10	<10	10	<10	<10	<10	<10	<10	<10
First Beach	<10	52	<10	<10	<10	124	10	42	99	<10	<10	<10	<10
Second Beach	<10	<10	<10	<10	<10	99	10	20	<10	<10	<10	<10	<10
Third Beach	31	110	10	31	<10	64	<10	150	<10	20	10	<10	11

Any result above the state standard is considered unsafe, and swimmers should refrain from swimming until results return to acceptable levels, or at least for several days after heavy rain. * - snow storm delayed February collection



STORM EVENT SAMPLING

- Sampling at the first stages of a major rainstorm
 - Shows the worst case scenario of site bacteria levels
 - Helpful in identifying sources of bacteria not readily seen during dry sample events
- Most effective if samples are collected within the first hour of a storm
- $>1/4$ " rainfall is often enough to elevate bacteria levels
- Storm event sampling can be a part of any plan



BEFORE HEADING OUT

- Let someone know you are heading out and when you expect to be back
 - If possible, have a co-sampler join you
- Check the weather
 - Dress appropriately
- Inspect equipment
 - Check condition of coolers, sample bottles, marker, sample bucket, etc.
 - Have backup sample bottles
 - Have safety equipment (first aid kit, flares, etc.)



IF SAMPLING ALONG A ROAD

- If a safe parking area away from the road is not available, pull completely off the road and onto the shoulder
 - Park away from sharp curves
 - Leave sufficient room to exit/enter the vehicle
 - Turn on the vehicle hazard signal
 - If possible, wear a reflective or orange safety vest
 - Try to exit away from the path of motor vehicles



BRIDGE SAFETY

- Be aware of traffic conditions
- If there is a no loitering or fishing sign, best not to sample on the bridge
- Sample on the upstream or the safest side of the bridge



CONTAMINATED WATER

- Use extra protection if water has an unusual odor or color
- Wear gloves or have hand sanitizer available during sampling
 - Remember you can contaminate the sample too!
- Wash or disinfect hands after sampling and prior to eating
- Always assume sample water contains pathogens!





Exercise Time

