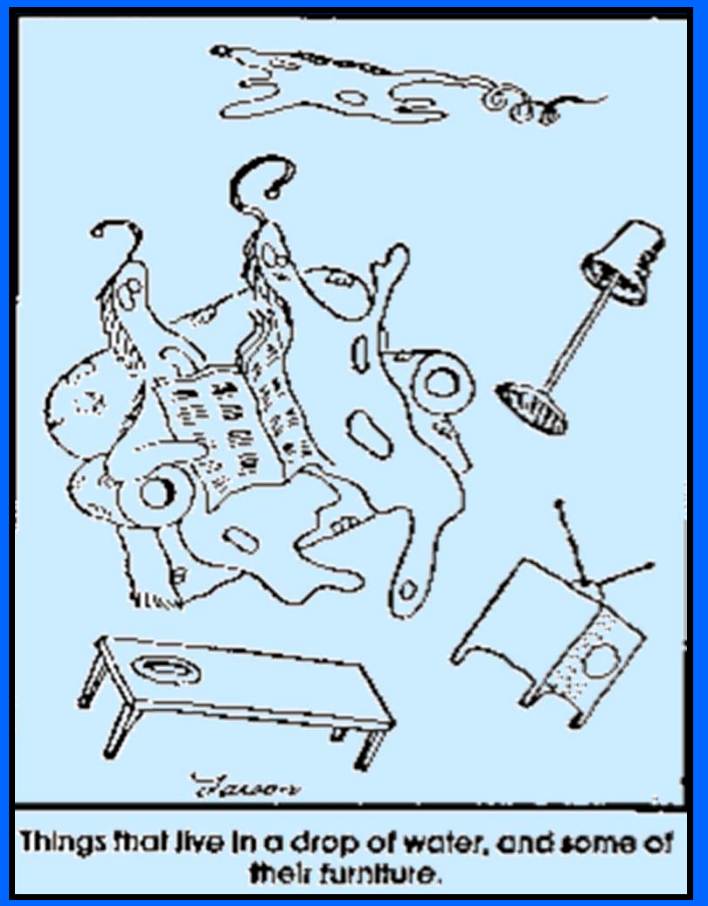
Bacteria 101 - Scope

- Bacteria Background
- Sources of fecal bacteria
- Indicator Organisms
- Pathogens
- Health risks
- Standards for bacteria



Why Monitor Bacteria?

- **Nearly 40% of assessed (700,000 miles) rivers and streams impaired by one or more uses[1]**

- **Bacteria/Pathogens identified as a major cause of water-quality impairment**
  - **93,431 river and stream miles**
  - **~ 3 million lake acres (23 % of assessed)**
  - **3,245 square miles of estuarine waters (15% of assessed)**

- **Ecological and health implications**

[1]2000 National Water Quality Inventory, U.S. Environmental Protection Agency, August 2002

Bacteria

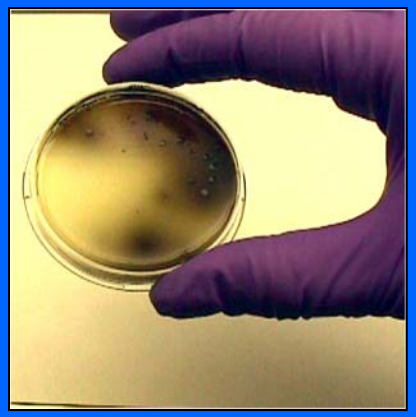
- **Unicellular organisms (procaryotes)**
- **Microscopic**
- **Can live in all types of environments**
- **Many bacteria beneficial**
- **Some bacteria of concern**

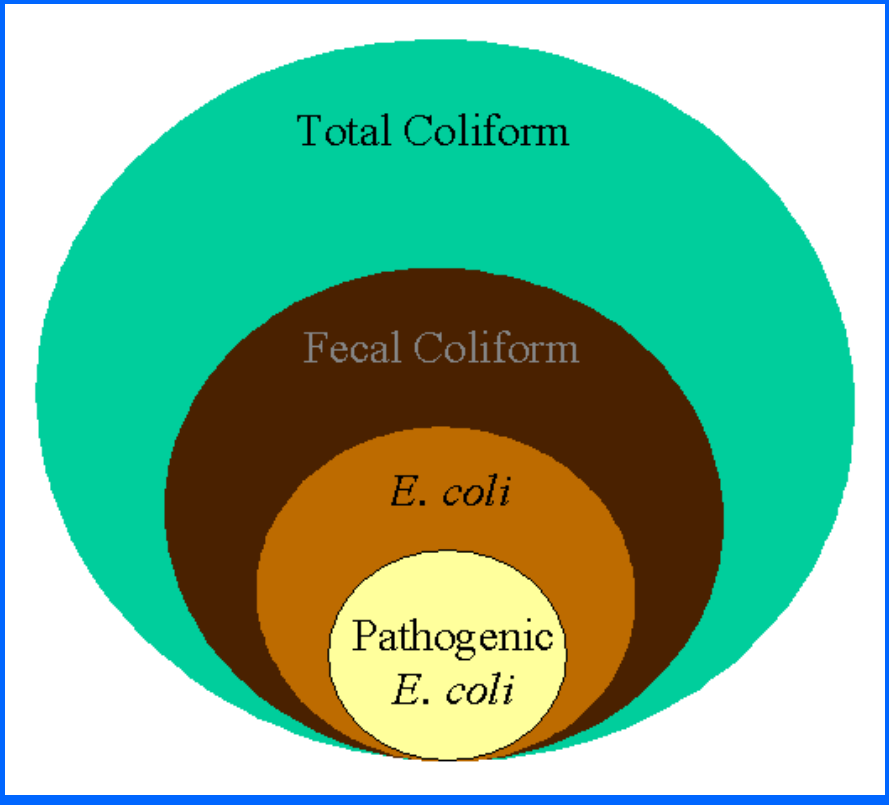
Focus today:

- *E. coli* and enterococci bacteria and their role as an indicator of the presence of pathogenic organisms

Fecal Coliform Bacteria

- Bacteria from feces of warm-blooded animals
- Most are nonpathogenic
- Present in higher number than pathogens



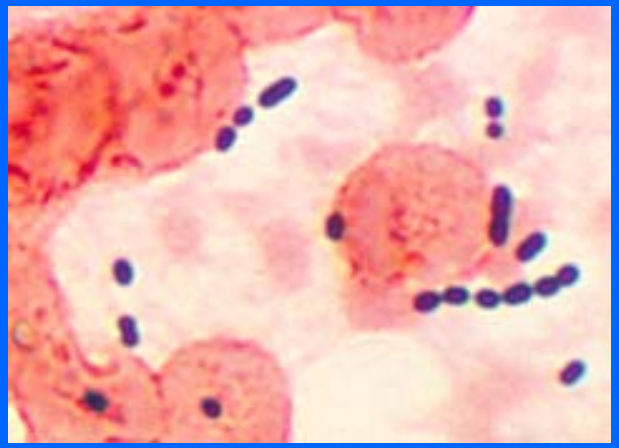


Coliforms

- gram negative, non-spore forming rod shaped bacteria which ferment lactose

Enterococci Bacteria

- Bacteria from feces of warm-blooded animals
- Separate bacterial group from coliforms
- Formerly classified as a fecal streptococcus
- EPA concluded were better than coliforms as indicators of pathogens causing gastrointestinal illness to swimmers in marine waters, useful in freshwater too.



Enterococci
- gram positive, sphere shaped lactic acid bacterium

Sources of fecal bacteria

- Human Sources – anytime fecal matter reaches water there will be bacteria
  - Wastewater treatment – inadequate or leaky septic systems or discharge from municipal systems
  - Swimming *"accidents"*, diapers
  - Boat dumping, water recreation

More Bacteria Sources

- Animal sources
  - Livestock – in streams, manure applied to fields, manure pits or lagoons
  - Pets
  - Wildlife – geese, ducks, deer, etc.

Indicator bacteria survival in environment

- Sunlight (UV radiation)*
  - *Turbidity*
- Temperature
- In sediment**
- In algal mats

Water body conditions that enhance survival

- *low light penetration, high turbidity, low salinity, presence of elevated nutrients and organic matter*

*Davies-Colley et al. 1994 Appl Environ Microbiol. 60 (6):2049-2058
**Jamieson et al. 2005 J. Environ. Qual. 34:581-589

Persistence in the environment

(Academy Creek–Brunswick, GA)

Condition	Enterococci (MPN) – Fecal Strep Bacteria
	Colony-forming units g^{-1} of dried sediment
Moist sediment	3,160
Dried 2 days and rewet	16,980
24 h after rewet	23,440
Dried 30 days and rewet	510
24 h after rewet	16,980
Dried 60 days and rewet	1,200
24 h after rewet	28,840

**Provided by Peter Hartel*

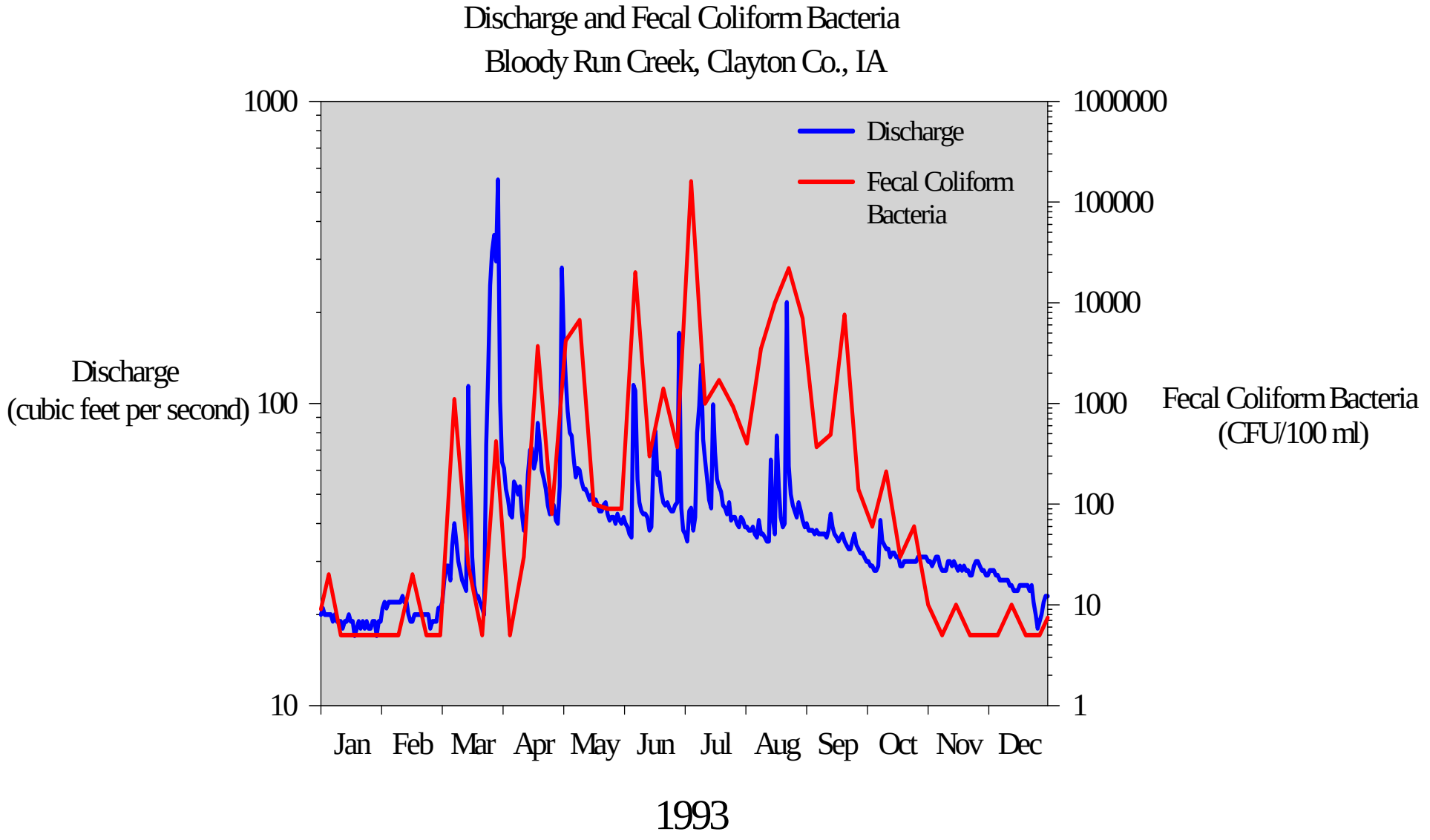
Bacteria levels are also affected by:

- Source and amount of loading
- Rainfall and runoff*

*Hill et al. 2006. Int. J. Environ. Res. Public Health 3(1), 114-117

Bacteria levels can be related to flow:

More runoff = Higher bacteria counts



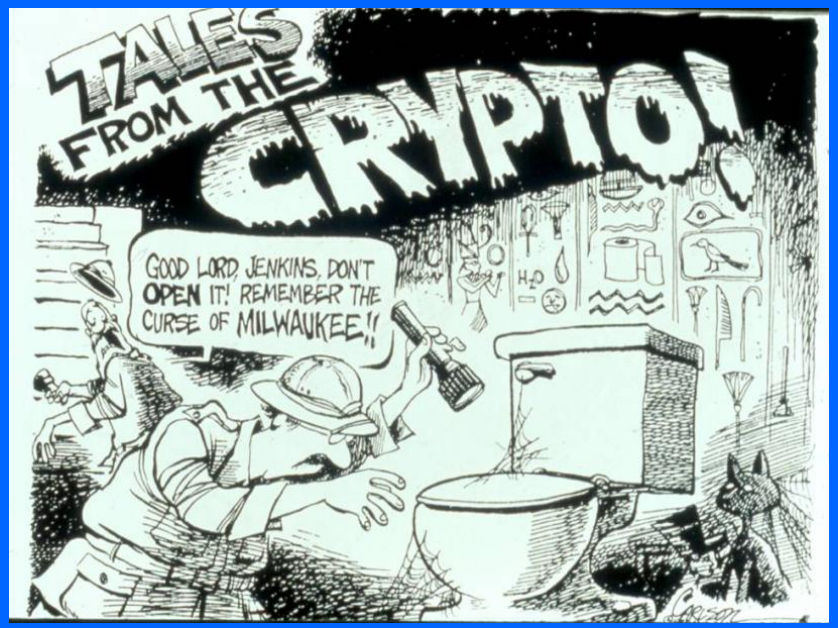
E. Coli & enterococci are used as indicators because they:

- Indicate recent fecal contamination
- Suggest the presence of pathogens

- Are easy to collect and analyze
- Are relatively safe to handle and generally harmless

Pathogens

- Pathogens are disease causing microorganisms which cause a variety of illnesses; they can be
  - Virus
  - Bacteria
  - Fungus
  - Parasites
- Symptoms of waterborne pathogen illness sometimes confused with other diseases



Some Bacterial Pathogens

Table 2-1. Pathogenic bacteria of concern to water quality and their associated diseases

Bacteria	Disease	Effects
Escherichia coli 0157:H7 (enteropathogenic)	Gastroenteritis	Vomiting, diarrhea
Salmonella typhi	Typhoid fever	High fever, diarrhea, ulceration of the small intestine
Salmonella	Salmonellosis	Diarrhea, dehydration
Shigella	Shigellosis	Bacillary dysentery
Vibrio cholerae	Cholera	Extremely heavy diarrhea, dehydration
Yersinia enterolitica	Yersinosis	Diarrhea

From USEPA 2001, Protocols for Developing Pathogen TMDLs. EPA 841-R-00-002.

Why not just sample for pathogens?

- There are many different pathogens
- Few laboratories have the capacity
- Costs
- Time
- Water volume
- Most tests identify only one pathogen
- Pathogenic organisms - difficult to isolate and identify

Minimal Infective Doses in Some Organisms

Organism	Minimal Infective Dose
Salmonella spp.	10,000 – 10 million
Shigella spp.	10 - 100
E. coli	1 million – 100 million
E. coli O157:H7	<100
Vibrio cholerae	1000
Campylobacter jejuni	~500
Giardia lamblia	10 -100 cysts
Cryptosporidium	10 oocysts
Hepatitis A virus	1 -10 pfu

From: Marylynn V. Yates, Department of Environmental Sciences, University of California, Riverside

From Bitton, 2006

E. coli Body contact standard

- Indicator of potential health risks from body contact (swimming)
- Varies by state – check YOUR state's standards
- EPA single sample standard is 235 cfu per 100 ml for swimming beach advisories
- Geometric Mean shall not exceed 126 *E. coli* / 100ml

Enterococci Body contact standard

- Varies by state – check YOUR state's standards
- EPA single sample standard is 104 cfu /100 ml for marine beach advisories, 62 cfu/100 ml for freshwater beach advisory
- Geometric Mean shall not exceed 35 cfu /100 ml for marine beach advisories, 33 cfu/100 ml for freshwater beach advisory

Geometric Mean

Method recommended by EPA. Based on 5 samples collected over a 30-day period. Minimizes influence of a one-time high result.

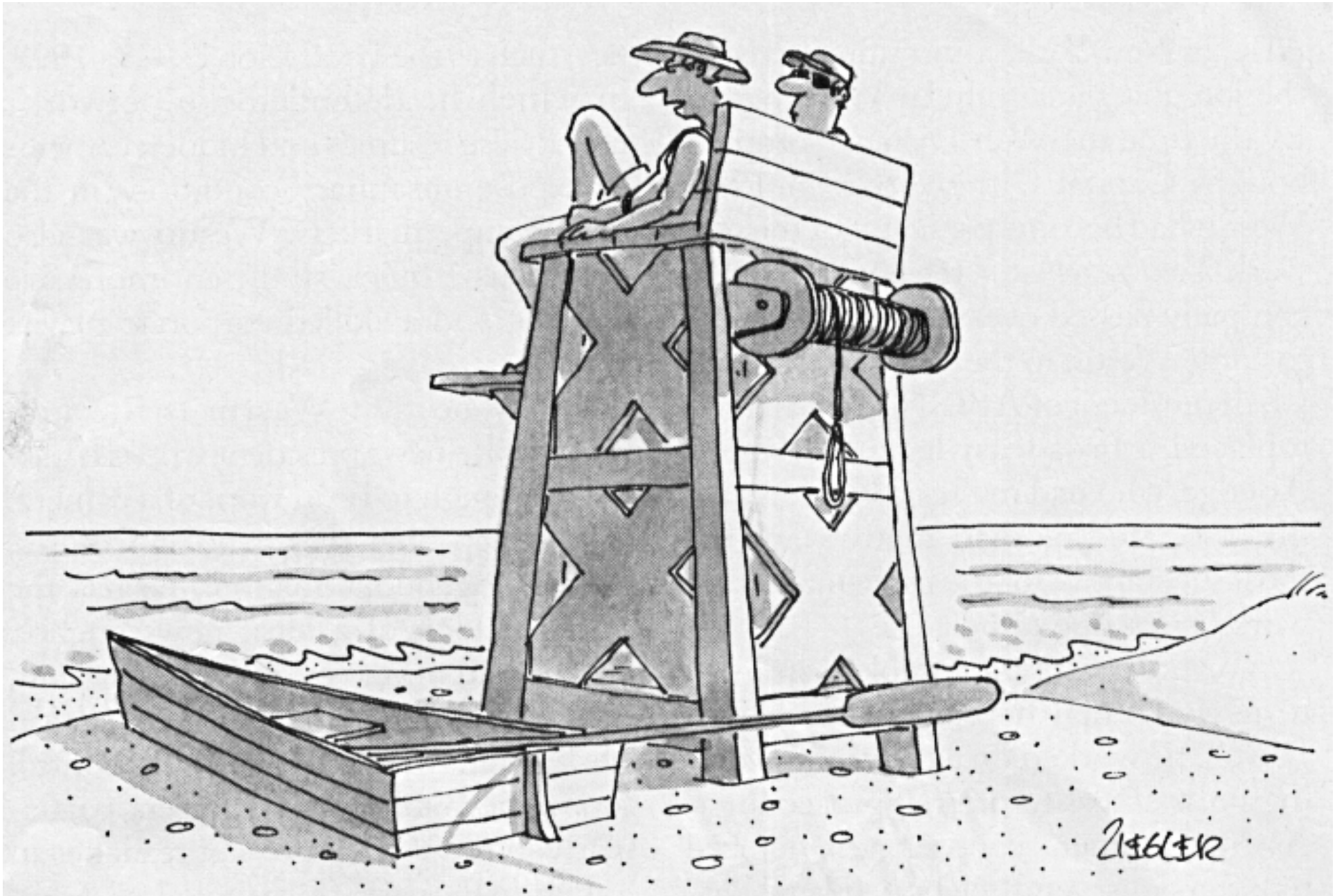
Example: Sunshine Lake with bacteria readings of 5, 10, 120, 20, 2700

Average would be $= \frac{5 + 10 + 120 + 20 + 2700}{5} = 571$

GM $= \sqrt[5]{5 * 10 * 120 * 20 * 2700} = 50$

Current Monitoring Approach Leads to Errors

Courtesy Richard Whitman - USGS



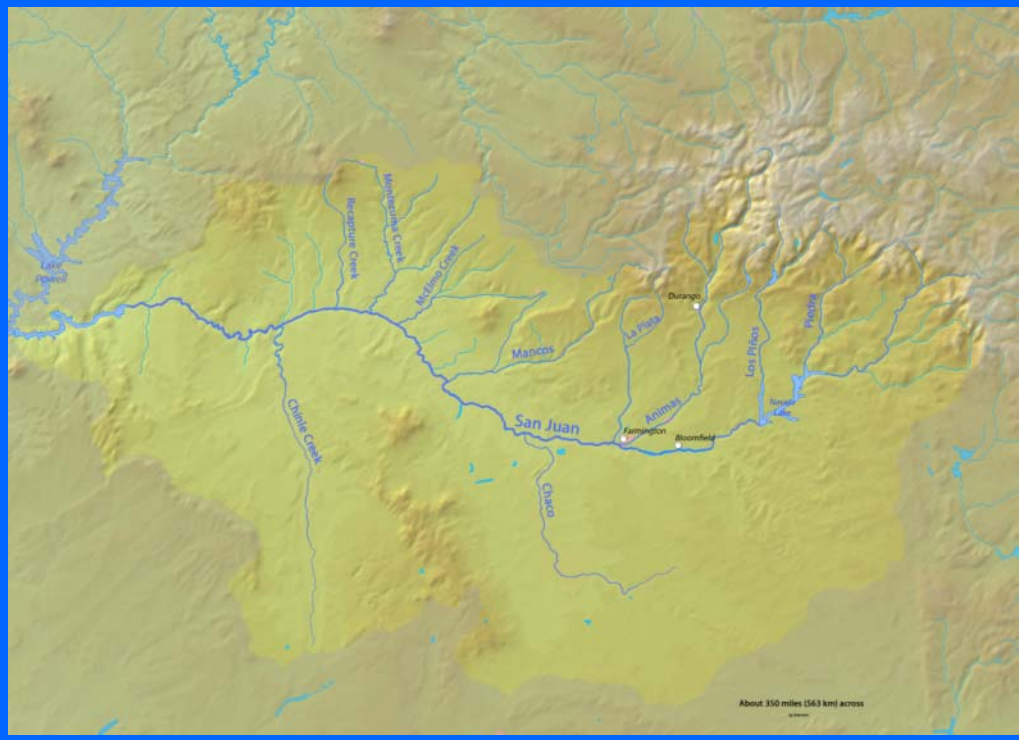
"I adore the beauty and tranquillity of these raw-sewage days."

Equipment

- Field list provided (p.11 manual)
- Suppliers list (provided)

Site Selection

- Choose sites based on project goals
- Consider
  - Tributaries or other inflows to the stream
  - Land uses alongside the stream
- Also consider:
  - Access rights
    - Know tribal and state laws
  - Budget
  - Number of volunteers
  - Safety



Site Selection Resources

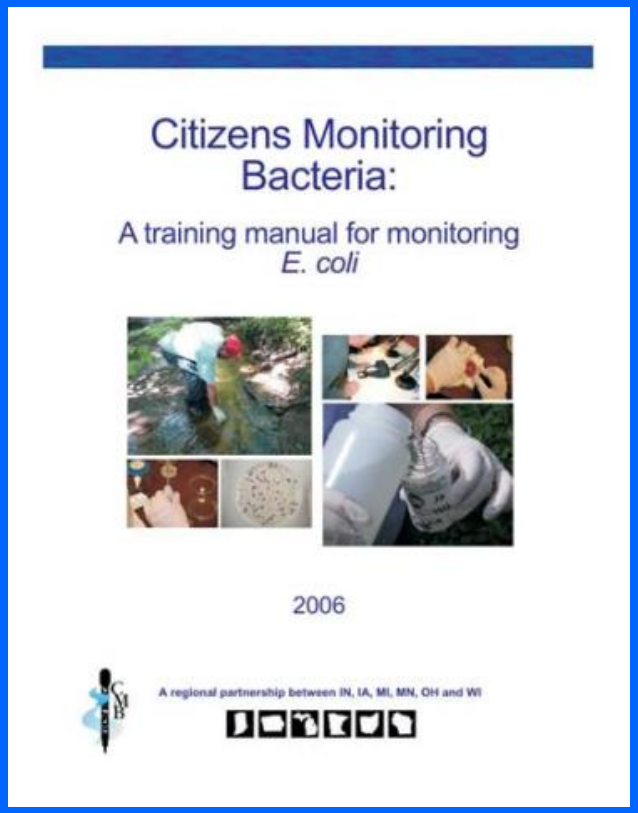
- EPA's Volunteer Stream Monitoring Methods Manual
  http://www.epa.gov/volunteer/stream/stream.pdf
- Washington Dept. of Ecology's, "A Citizen's Guide to Understanding and Monitoring Streams and Lakes"
  http://www.ecy.wa.gov/Programs/wq/plants/management/joysmanual/selectingstreamlocations.html
- Citizen's Guide to Bacteria Monitorign in Vermont Waters
  http://www.vtwaterquality.org/lakes/docs/lp_citbactmonguide.pdf

Frequency of Monitoring

- Choose frequency based on project goals
  - Check your local recommendations
  - Monthly for screening
  - Five times within 30 days to determine geometric mean
- Be consistent in time of day
- Have regular intervals between sampling dates

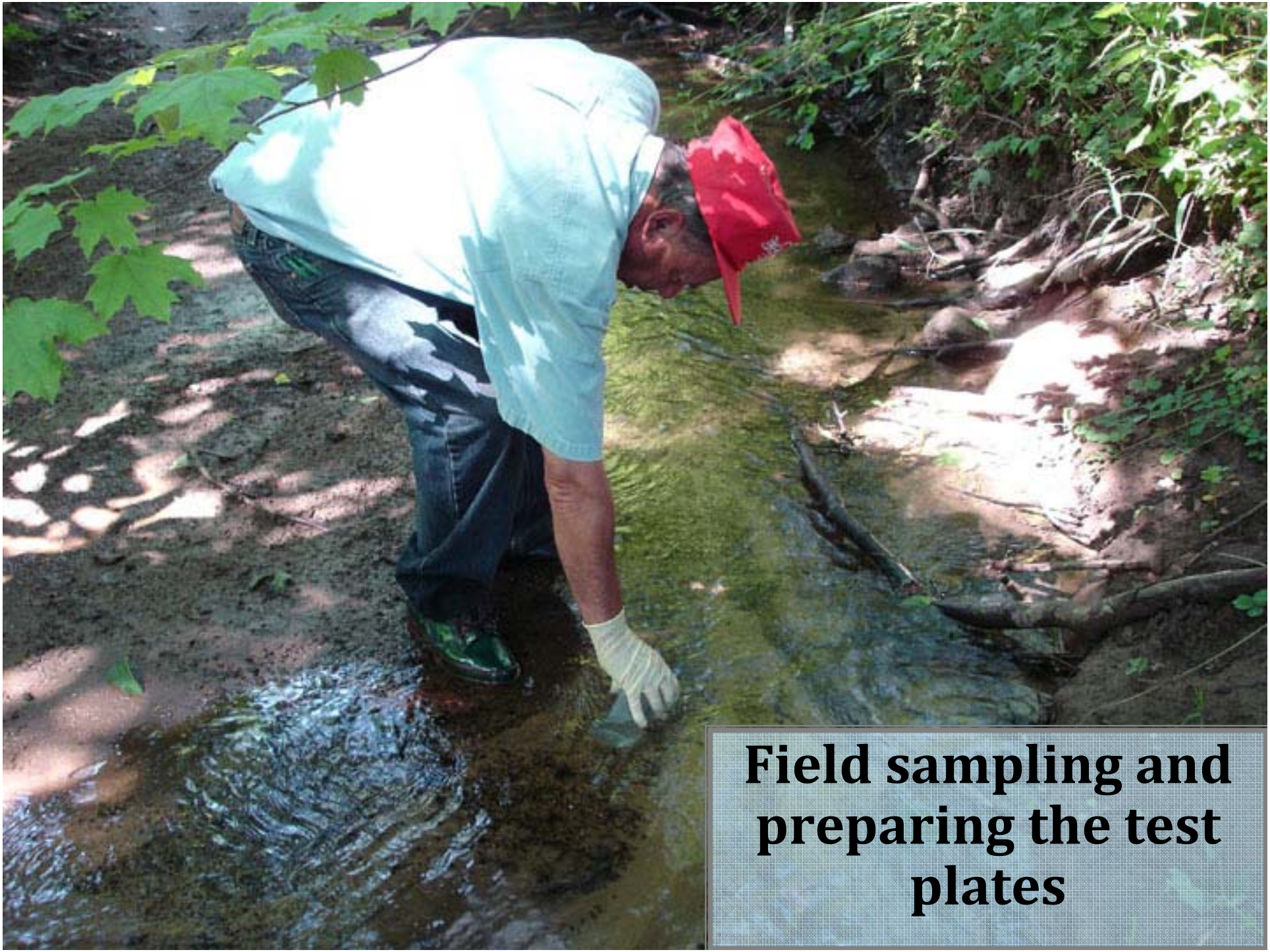
Quality Assurance (p. 14)

- Maintain quality in practices and procedures
  - Defining and documenting methodologies
  - Training volunteers
  - Keeping accurate records
  - Checking expiration dates



Quality Control (p.15)

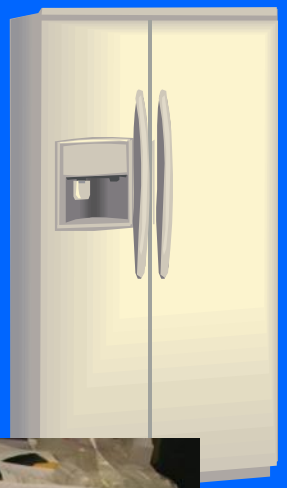
- Ensure samples are collected consistently and accurately
  - No contamination of sample
  - Field blanks – DI H_2O into sterile bottles at 10% sites
  - Field replicates – 2nd sample collected at 5-10% of sites
  - Lab replicates – Plate 2 or more samples from 1 bottle
  - Control plates – Plate DI H_2O to ensure no lab contamination
  - Split samples – e.g., splitting a sample and sending to 2 labs
  - Regular inspection of equipment

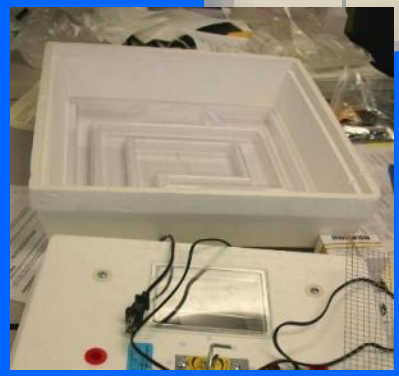


Field sampling and preparing the test plates

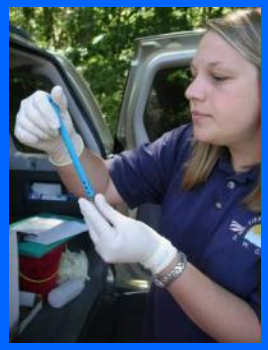
Field Sampling – Before you go

- Take Petrifilm out of fridge to warm to room temperature
- Fill trays in incubator with distilled water
- Turn on incubator to 35°C
- Collect supplies, including ice (preferred) or an ice pack

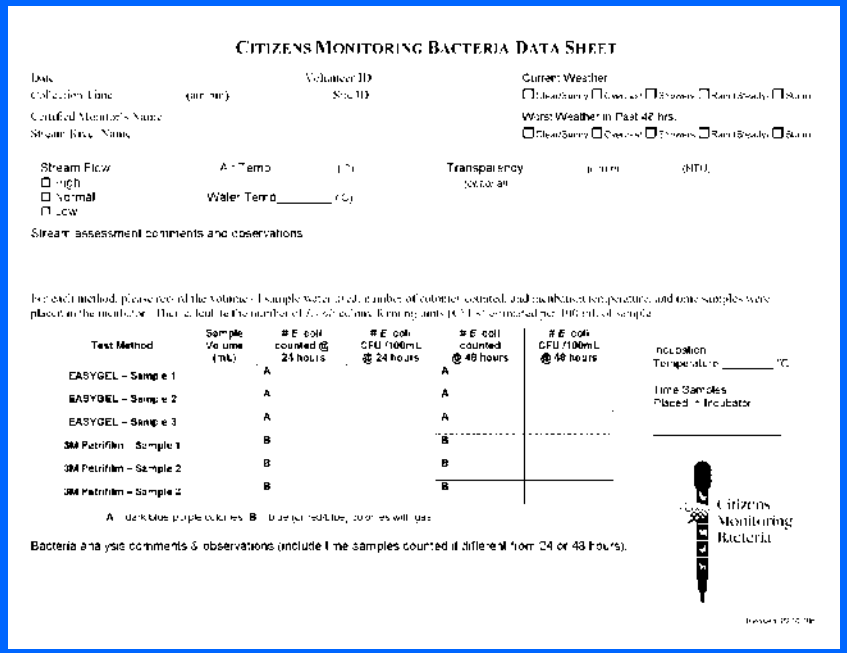




Field Sampling at the Site



- Hang thermometer for air temp
- Complete top section of data sheet
- Take water temp, record on data sheet
- Put on gloves
- Label sample bottles
- Take transparency tube reading (optional)



CITIZENS MONITORING BACTERIA DATA SHEET

Date
Collection Time (am/pm)
Volunteer ID
Site ID
Current Weather
☐Clear/Sunny ☐Cloudy ☐Showers ☐Rain/Steady ☐Storm
Certified Monitor's Name
Stream/Bay Name
Worst Weather in Past 48 hrs.
☐Clear/Sunny ☐Cloudy ☐Showers ☐Rain/Steady ☐Storm

Stream Flow
☐ High
☐ Normal
☐ Low

Air Temp (°C)
Water Temp_____(°C)
Transparency
tube or all
(cm) (NTU)

Stream assessment comments and observations

For each method, please record the volume (sample water tested), number of colonies counted, and incubation temperature and time samples were placed in the incubator. Then calculate the number of E. coli colonies forming units (CFU) per 100 mL of sample.

Test Method	Sample Volume (mL)	# E. coli counted @ 24 hours	# E. coli CFU /100mL @ 24 hours	# E. coli counted @ 48 hours	# E. coli CFU /100mL @ 48 hours
EASYGEL – Sample 1		A		A	
EASYGEL – Sample 2		A		A	
EASYGEL – Sample 3		A		A	
3M Petrifilm – Sample 1		B		B	
3M Petrifilm – Sample 2		B		B	
3M Petrifilm – Sample 3		B		B	

A dark blue purple colonies B Blue gas bubble, some yellow gas

Bacteria analyst comments & observations (include if the samples counted if different from 24 or 48 hours).

Incubation Temperature _____ °C

Time Samples Placed in Incubator

_____

Citizens Monitoring Bacteria

Collecting a Sample

1. *Wading*: Try to disturb as little bottom sediment as possible. In any case, be careful not to collect water that has sediment from bottom disturbance. Wade to the depth where most users typically swim. *Boat or dock:* Carefully reach over the side and collect the water sample away from the side of the boat or dock and at the depth where most users swim.
2. Remove the cap from a sterile collection bottle without touching the inside of the cap or the inside of the bottle.
3. Grip the bottle at the base and plunge it in a downward motion into the water to a depth of 12 to 18 inches.
4. Using a forward sweeping motion (so water is not washed over the hand into the bottle), invert the bottle and bring it to the surface.
5. Empty it slightly to leave approximately one inch of air at the top.
6. Re-cap the container, then label and store it on ice at a temperature between 39° and 45° F. It is better to use wet ice rather than cold packs.
7. Transport the bottle to the laboratory as soon as possible after sampling.

Accuracy and variability

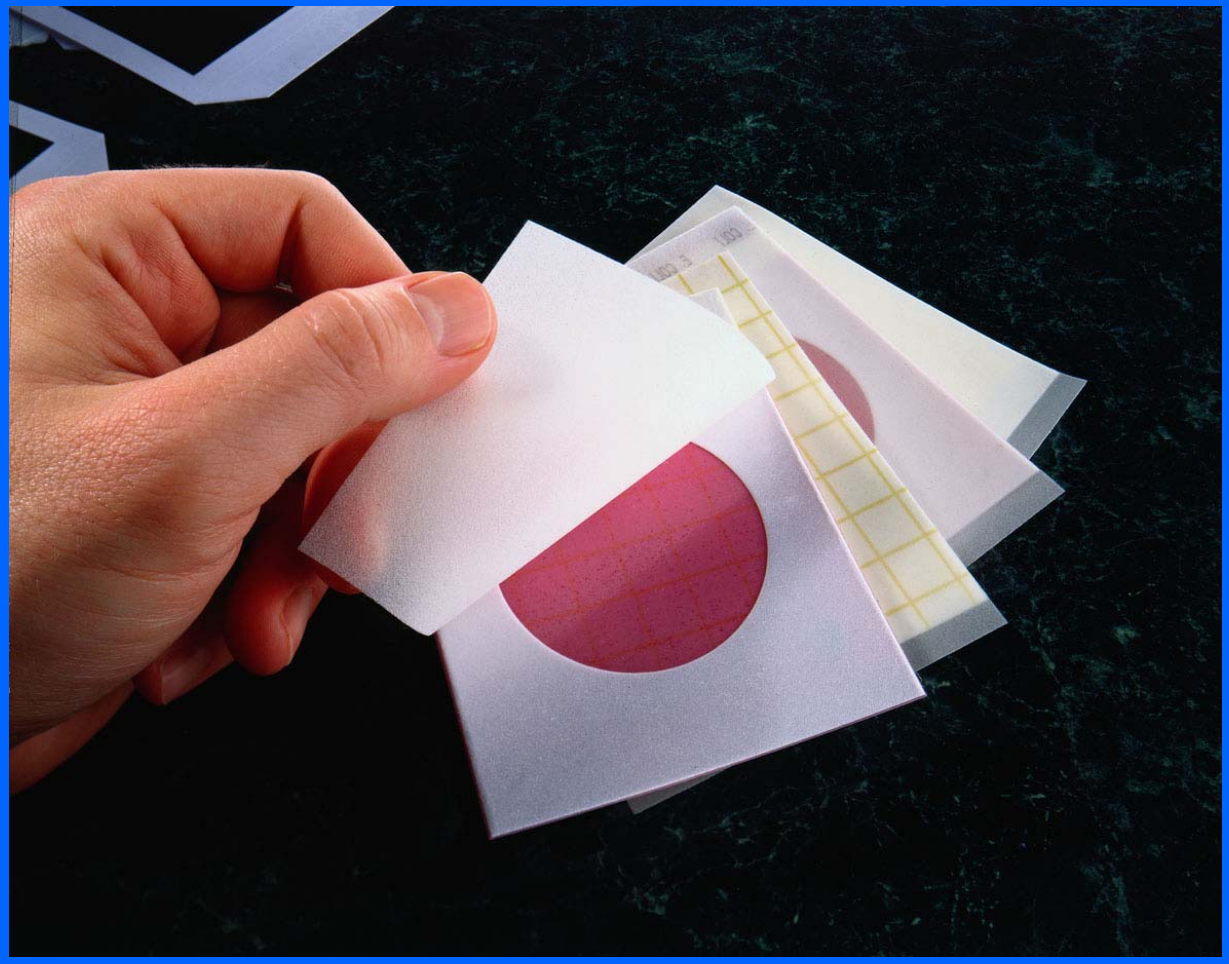
Results may not be accurate when:

- Samples aren't kept cold
- Samples don't reach lab within 24 hours

- Samples aren't from a single split
- Work space or equipment isn't sterile
- Bottles, lids, pipettes are contaminated

Time for Some Hands-On Work

3M™ Petrifilm™



Preparing to plate samples

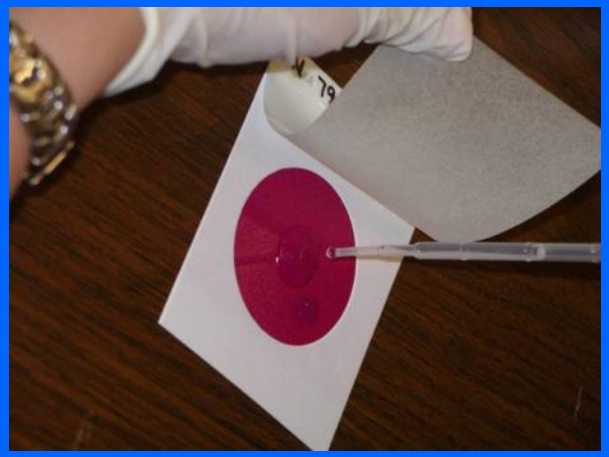
- Disinfect work space and collect supplies
- Check incubator temp, adjust to 35°C
- Make sure Petrifilm plates are at room temperature

Preparation continued

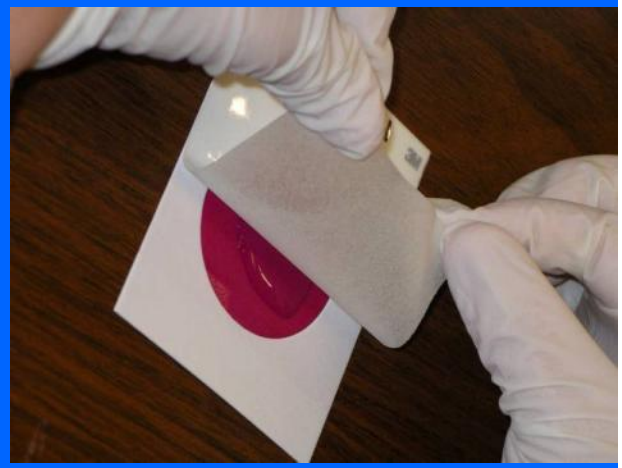
- Label the back of Petrifilm using a permanent marker
  - Label should include:
    - Site name
    - Date of collection
    - Replicate number
- Note the incubation temperature and time that samples were placed in incubator

Plating 3M™ Petrifilm™

1. Pipette water sample (1 ml only)



2. Lift film and dispense sample onto pink



3. Carefully roll down film

4. Gently use spreader, if needed

5. Let sit 1 minute

6. Put in incubator right side up (can be stacked)

IDEXX

- Colilert
  (Coliform/E. coli 24 hrs)
- Colilert-18
  (Coliform/E. coli 18 hrs)
- Enterolert
  (Enterococci in 24 hrs)

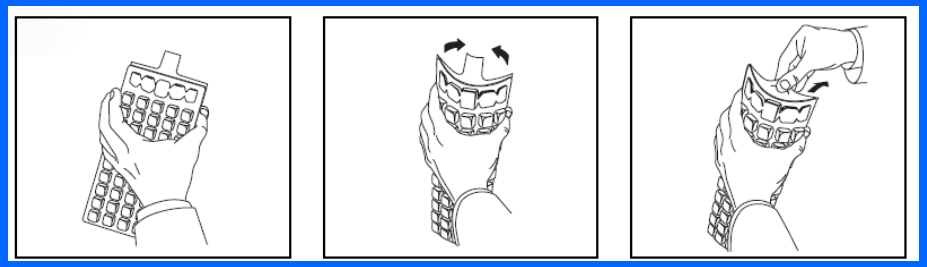
Preparing IDEXX samples

- Pour sample into mixing container
  (add sterile water to bring volume up to 100 ml if sample dilution is necessary)
- Pour media (Colilert, Colilert-18 or Enterolert) in the mixing container
- Shake well, allow foam to settle

Label the Quanti-Tray with sample ID and volume info

Filling the Quanti-Tray

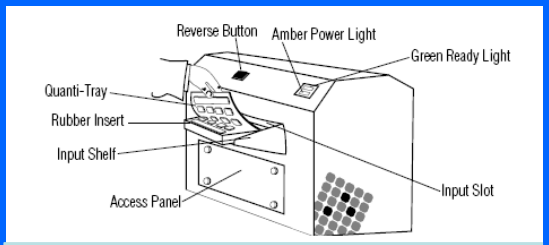
1. Use 1 hand to hold a sterile Quanti-Tray upright with the well side facing the palm
2. Squeeze the upper part of the tray, carefully pull tab so the tray opens (Do not touch the inside!!)
3. Pour the reagent/sample mixture into the tray
4. Tap the tray to help remove bubbles – allow any foam to settle



Detailed instructions available at:
http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/cwt/guidance/3410.pdf

Sealing the Quanti-Tray

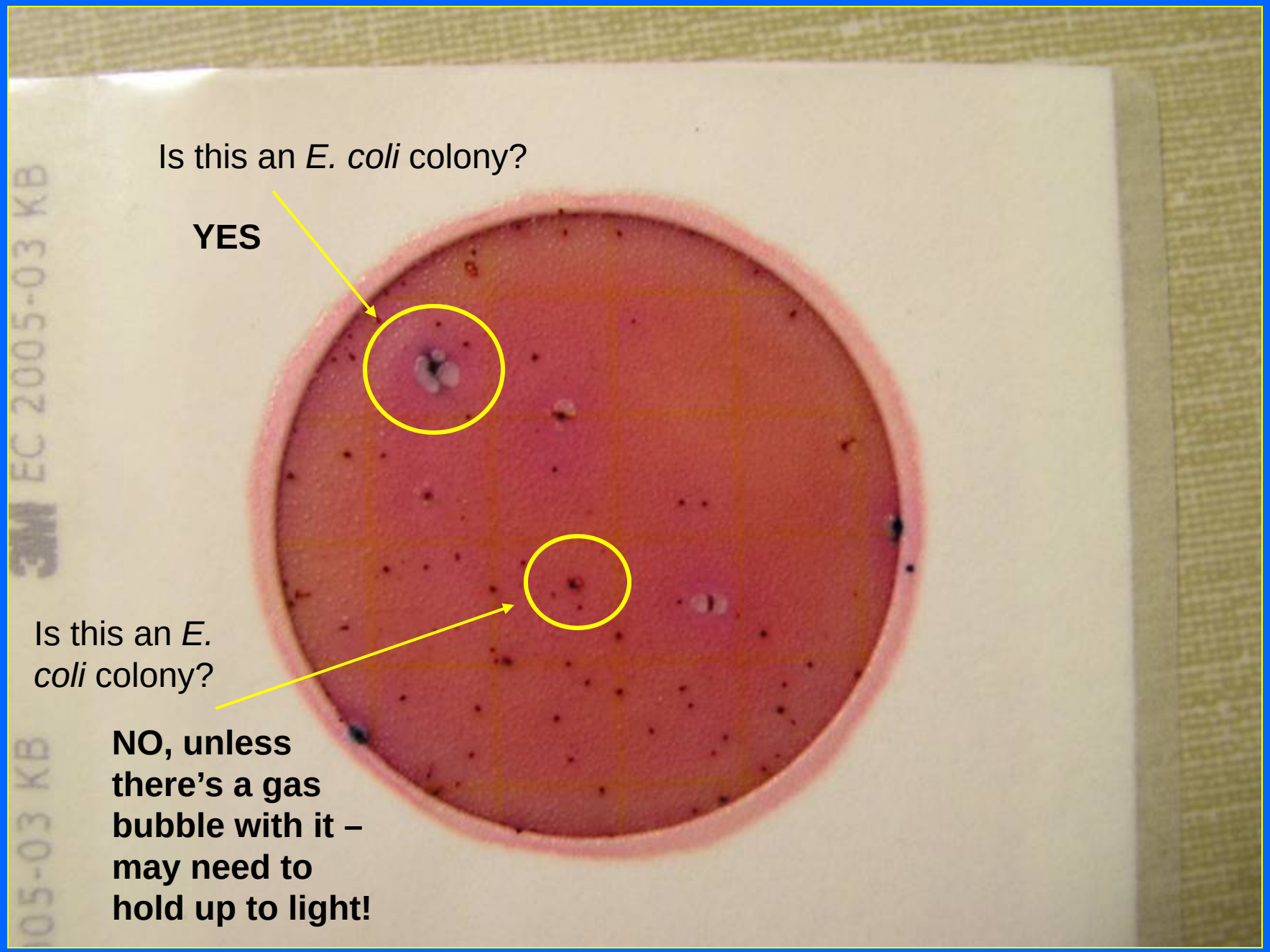
1. Set the Quanti-Tray in the proper rubber insert.
2. Place the Quanti-Tray/Insert onto the input shelf and feed it into the sealer.
3. Remove Quanti-Tray /Insert. If they were fed crookedly, stop and reverse, and reinsert .
4. Incubate trays
   - Colilert : 35 °C for 24 hrs
   - Colilert-18: 35 °C for 18 hrs
   - Enterolert: 41 °C for 24 hrs



The Quanti-Tray Sealer

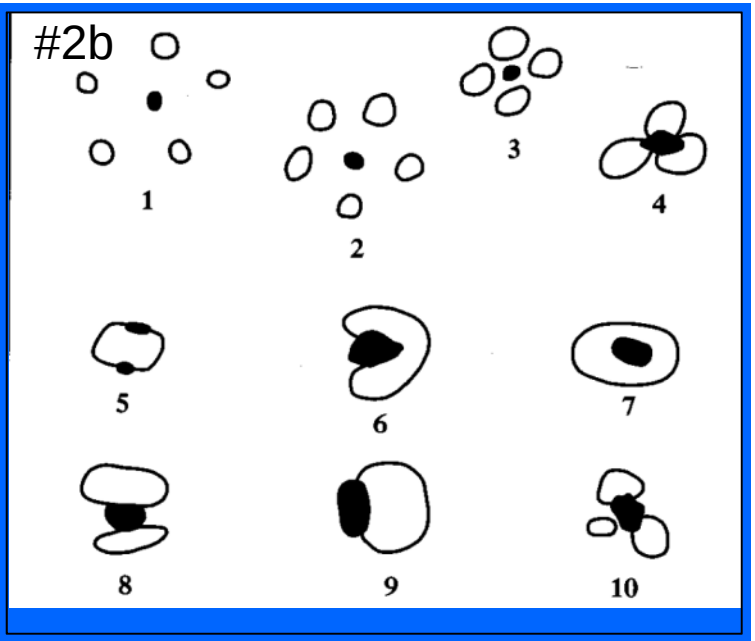
Reading & Interpreting Petrifilm Plates

- Count colonies after 24 hours incubation
- Count blue colonies WITH gas bubbles (these are *E. coli*)
- Don't count blue colonies without gas bubbles
- Don't count pink or white colonies with or without gas bubbles


Is this an *E. coli* colony?
YES
Is this an *E. coli* colony?
NO, unless there's a gas bubble with it – may need to hold up to light!
3M EC 2005-03 KB

#2a

#2b



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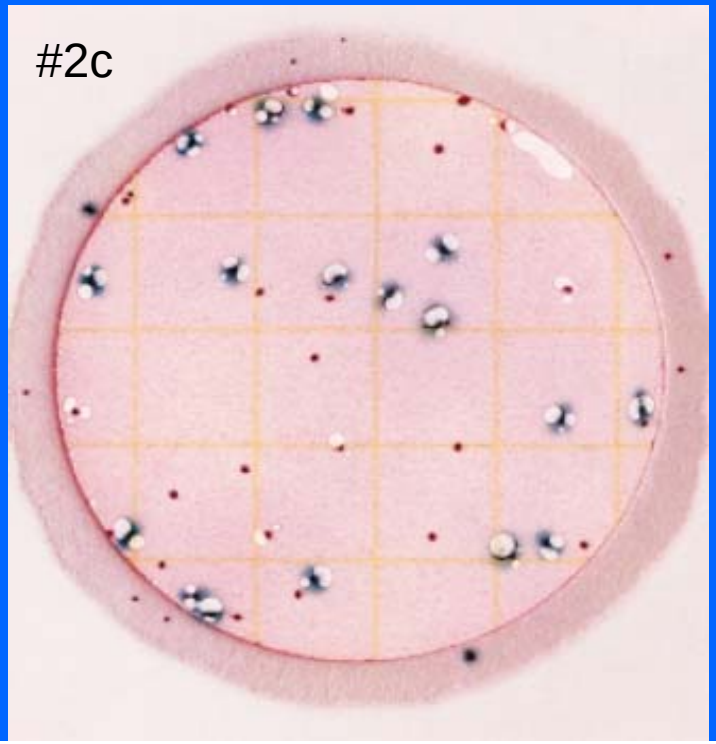
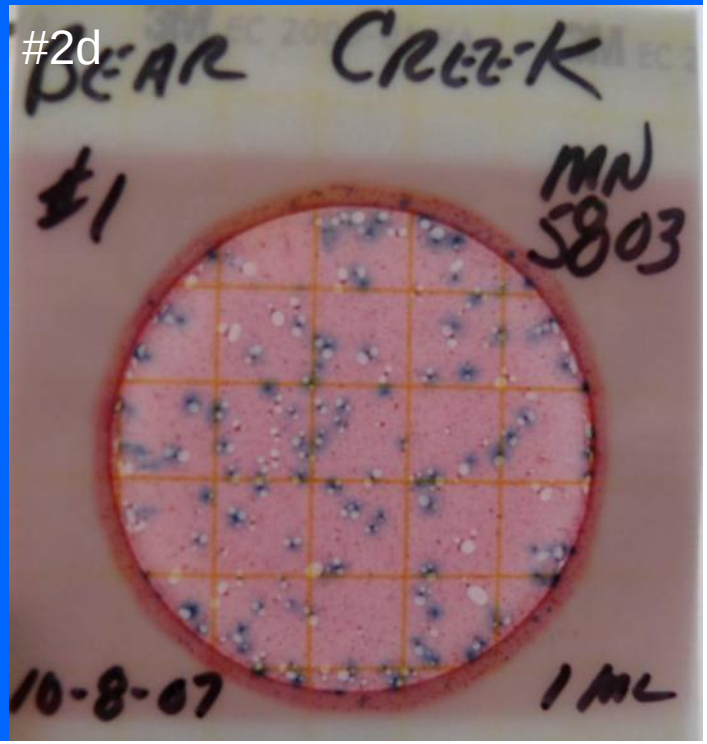


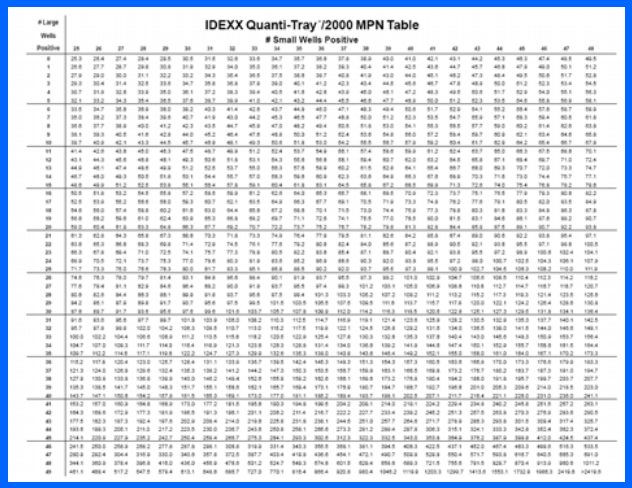
3M Petrifilm™ Sample Plates & Graphic

#2d



Preparing for Reading IDEXX

- Results Notebook/data sheets
- UV Lamp
- Anti-UV goggles or cabinet
- Quanti-tray MPN tables



IDEXX Quanti-Tray®/2000 MPN Table

Small Wells Positive

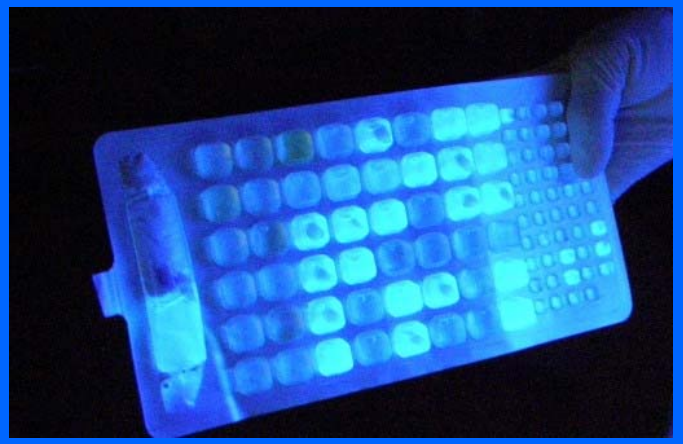
# Large Wells Positive	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.6	31.6	32.8	33.8	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.8	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.8	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.6	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.9	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.6	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.3	44.5	45.8	46.9	48.1	49.3	50.6	51.9	53.1	54.3	55.6	56.9	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.6	51.9	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.6	51.9	53.2	54.6	55.9	57.2	58.6	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.8	54.2	55.5	56.9	58.3	59.7	61.1	62.5	63.9	65.3	66.7	68.1	69.5	70.9	72.3	73.8	75.2	76.6	78.1	79.5	81.0	82.4	83.9	85.4
18	54.8	56.2	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.9	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.8	63.3	64.8	66.2	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.9	69.4	71.0	72.6	74.1	75.7	77.3	79.0	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.6	95.3	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.8
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.6	109.6	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.8	112.0	114.1	116.3	118.5	120.6	122.8	125.1	127.3	129.6	131.8	134.1	136.4
31	91.8	93.8	95.8	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	96.7	97.9	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.9	129.2	131.6	134.0	136.5	138.9	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.9	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.9	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.5	149.4	152.3	155.3	158.3	161.4	164.5	167.7	170.9	174.1
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.6	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.9	133.8	136.8	139.9	143.0	146.2	149.4	152.8	156.2	159.6	163.2	166.8	170.5	174.2	178.0	182.0	186.0	190.1	194.2	198.5	202.8	207.3	211.8
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.9	238.1	244.5	251.0	257.7	264.5	271.7	279.0	286.5	293.9	301.5	309.4	317.4	325.7
44	193.8	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.6	256.1	266.6	273.3	281.2	289.4	297.9	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.0	227.8	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.8	312.5	322.5	332.8	343.5	353.6	364.0	374.8	387.0	399.4	412.0	424.9	437.4
46	241.8	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.8	331.4	343.3	355.6	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.6	436.5	454.1	472.1	490.7	509.9	529.6	550.4	571.7	593.6	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.2	457.3	479.5	502.2	524.7	548.3	574.9	601.6	628.4	656.9	686.3	721.5	755.6	791.5	828.7	870.4	913.9	960.6	1011.2
49	451.1	469.4	517.2	547.5	579.4	613.1	648.8	686.7	727.2	770.1	815.4	866.4	920.8	980.4	1045.2	1116.9	1200.3	1299.7	1413.6	1553.1	1732.9	1985.3	2419.8	>2419.8

51-Well Quanti-Tray MPN Table

No. of wells giving positive reaction per 100 ml sample	Most Probable Number	95% Confidence Limits Lower	Upper
0	<1	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.5	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.9
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	367.6
51	> 200.5	146.1	Infinite

Reading IDEXX

- Count the yellow cells that are positive, marking the cell with a “Sharpie”.
- Using the cabinet or wearing anti-UV glasses/goggles, use a 6-watt 365nm UV light within 5 inches of the sample in a dark environment and count the positive (fluorescing) cells.
- Record results and compare to the MPN table



Disposing of plates

- Add a teaspoon of bleach to a ziplock bag
- Put Petrifilm plates in the ziplock bag
- Zip tightly and throw in trash
- Dump remaining water sample and throw sample bottle in rubbish

IDEXX Sample Disposal

- Since the Quanti-Trays are now filled with bacteria, after they have been read, they should be treated as hazardous waste.
- Used Quanti-Trays can be sterilized by putting them in an autoclave after which they can be treated as normal waste.
- See your state agency for specific regulations.

Calculate average of triplicates

Petrifilm:

- Simple average

mls Used	Colonies counted	Calculated cfu/100 mls
1	6	600
1	7	700
1	4	400

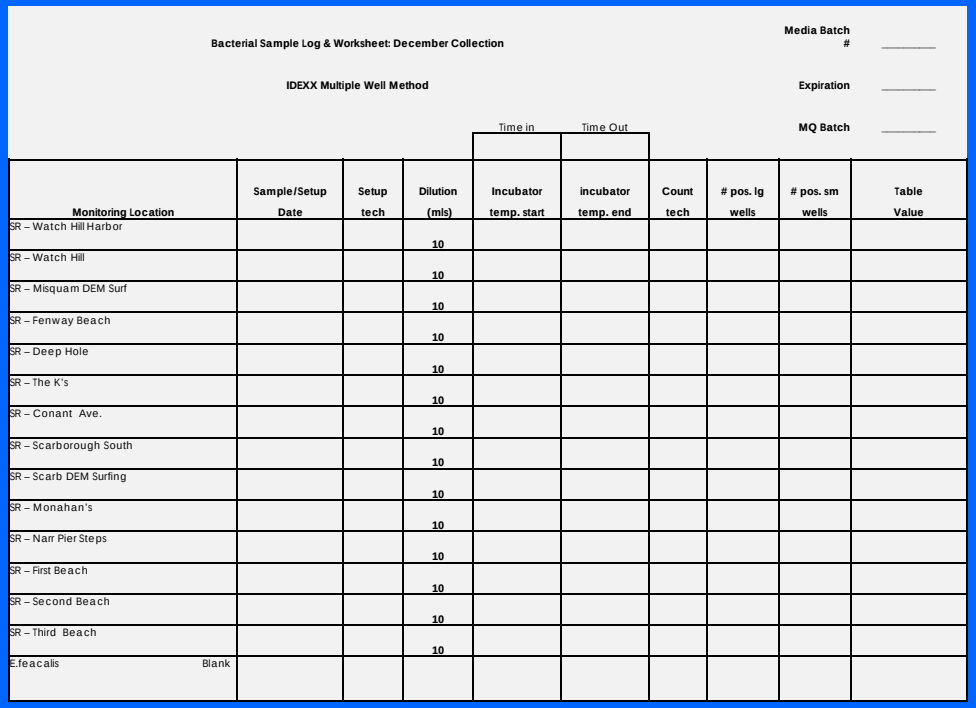
Average = (600+700+400) / 3

= 566.66 cfu/100 ml

IDEXX Datasheets

Should include space for:

- Sample Volume
- Incubation temps and time
- Reagent info
- Positive cells
- MPN Table values



Bacterial Sample Log & Worksheet: December Collection

IDEXX Multiple Well Method

Media Batch # _____

Expiration _____

MQ Batch _____

				Time in	Time Out				
Monitoring Location	**Sample/Setup Date**	**Setup tech**	**Dilution (mls)**	**Incubator temp. start**	**incubator temp. end**	**Count tech**	**# pos. lg wells**	**# pos. sm wells**	**Table Value**
SR – Watch Hill Harbor			10						
SR – Watch Hill			10						
SR – Misquam DEM Surf			10						
SR – Fenway Beach			10						
SR – Deep Hole			10						
SR – The K's			10						
SR – Conant Ave.			10						
SR – Scarborough South			10						
SR – Scarb DEM Surfing			10						
SR – Monahan's			10						
SR – Narr Pier Steps			10						
SR – First Beach			10						
SR – Second Beach			10						
SR – Third Beach			10						
E.feacalis Blank									

Questions??