IOWATER

Volunteer Water Quality Monitoring

Bacteria Monitoring

***E. coli* bacterium**

What you will learn during the bacteria workshop

- What are *E. coli* bacteria
- What sources of bacteria occur in Iowa
- How bacteria enter our waterbodies
- What health concerns are associated with bacteria
- How to monitor for bacteria
- How to interpret your bacteria results

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What are bacteria

- Bacteria are microscopic, single-celled organisms common in the environment
- Bacteria live in the air, soil, water, plants, and animals
- Most bacteria provide beneficial uses (aid in digestion; decompose dead plants and animals; process sewage sludge; aid in food production; digest hydrocarbons)
- Some bacteria are harmful and can cause health problems (disease causing bacteria are called pathogens)

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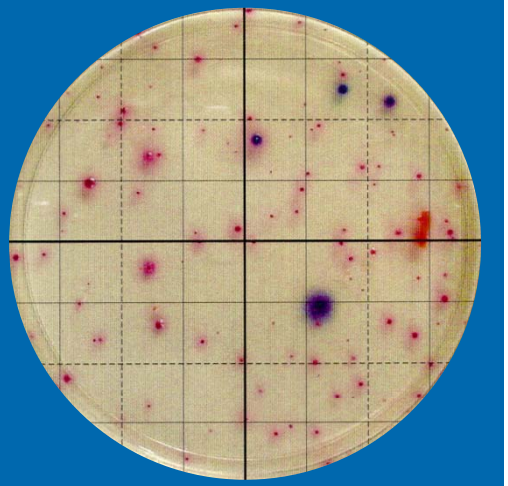
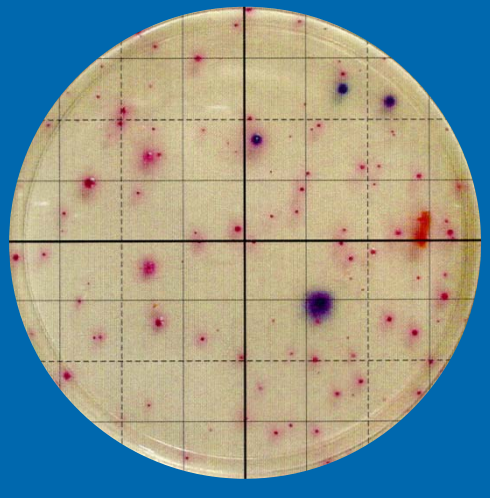
Indicator Bacteria



- Suggest a fresh source of feces
- Not necessarily harmful themselves
- Suggest the presence of pathogens



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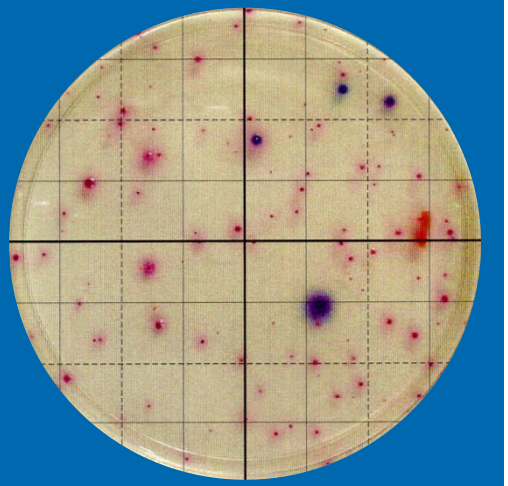
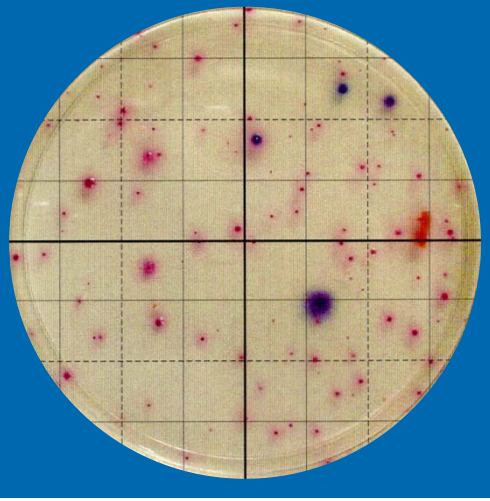
Indicator Bacteria and Pathogens



Indicator Bacteria Monitoring

Easy to monitor!



Pathogen Monitoring

Expensive Tests

Each pathogen requires separate test

Impractical

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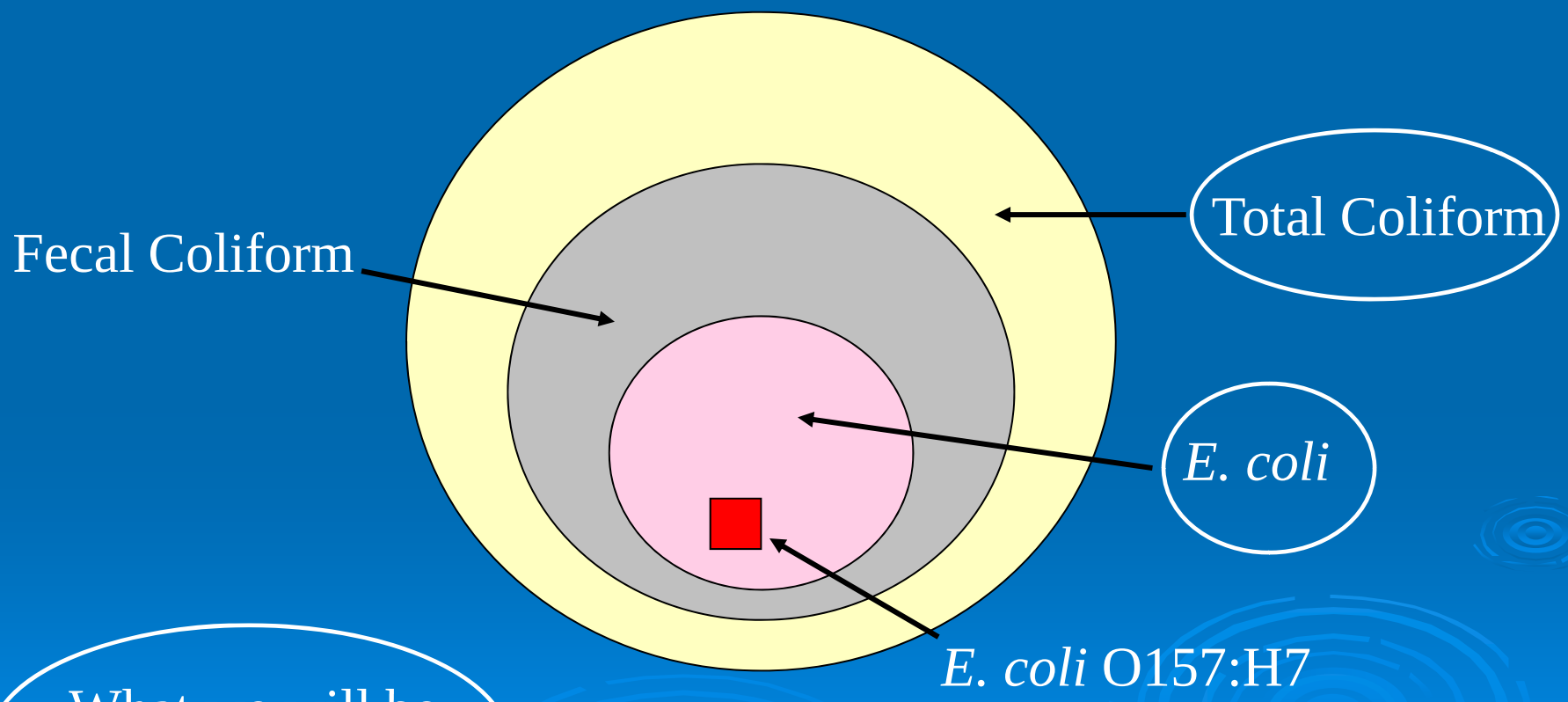
Coliform Group Indicators



What we will be monitoring for

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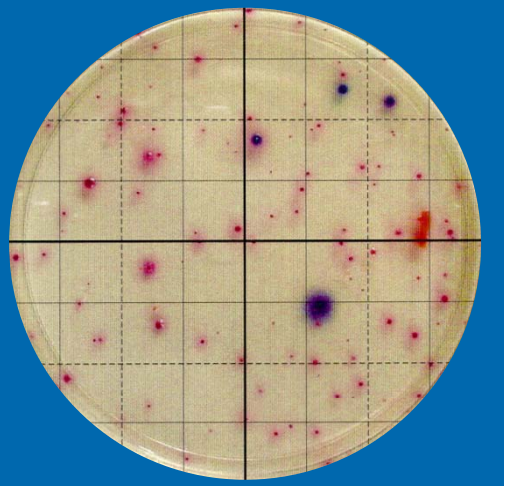
Pathogen – Greek for "birth of pain"



- *Salmonella* (Bacteria)
- Hepatitis (Virus)
- *Cryptosporidium* (Protozoa)
- *Giardia* (Protozoa)
- Pathogenic *E. coli* (Bacteria)

Monitoring for *E. coli* can be used to asses the likelihood of risks to human health from exposure to water containing *E. coli* bacteria

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BS1

E. coli 0157:H7

- There are hundreds of strains of *E. coli* bacteria
- *E. coli* O157:H7 is one particular strain of *E. coli*
- *E. coli* O157:H7 is primarily a foodborne pathogen
- Examples of foods contaminated with *E. coli* O157:H7 include ground meat, milk, spinach
- For bacteria testing as part of IOWATER, the presence of *E. coli* bacteria <u>does not mean</u> that *E. coli* O157:H7 is present

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Slide 8

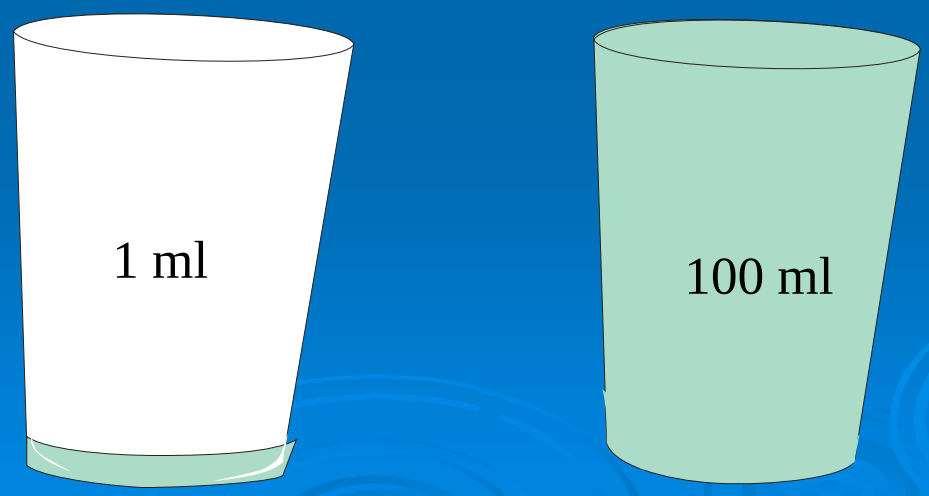
BS1 I've never really understood the E. coli 0157:H7 relationship. I suspect we make specific mention of it because of breakouts like Jack-in-the-Box and spinach that volunteers might associate with E. coli, but it also seems like we really empahsize that it's probably not present. While generally associated with contaminated food, isn't 0157:H7 still found in the intestines and passed in excrement? Wasn't the spinach outbreak caused by irrigation water contaminated with manure? If that's the case, isn't it somewhat probable that 0157:H7 could be found in fecally contaminated stream water? It seems to me like we go though great lengths to try to assure volunteers that they won't encounter it. Should we mention that this is a type of E. coli they could encounter and emphasize proper cleanup & safety measures?

BSoenen, 11/16/2010

BS2

How are bacteria levels measured

- Number of colonies of bacteria in your water sample is converted to the number that would be found in 100 milliliters of water (*Colony Forming Units per 100 ml of water – CFU/100 ml*)



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Slide 9

BS2 Should we move this slide down to the data interpretation section? It just seems a little out of place here...mabye after slide #17?
BSoenen, 11/16/2010

BS4

Sources and pathways for *E. coli* bacteria to enter water bodies

NRCS Photo

Iowa DNR Photo

NRCS Photo

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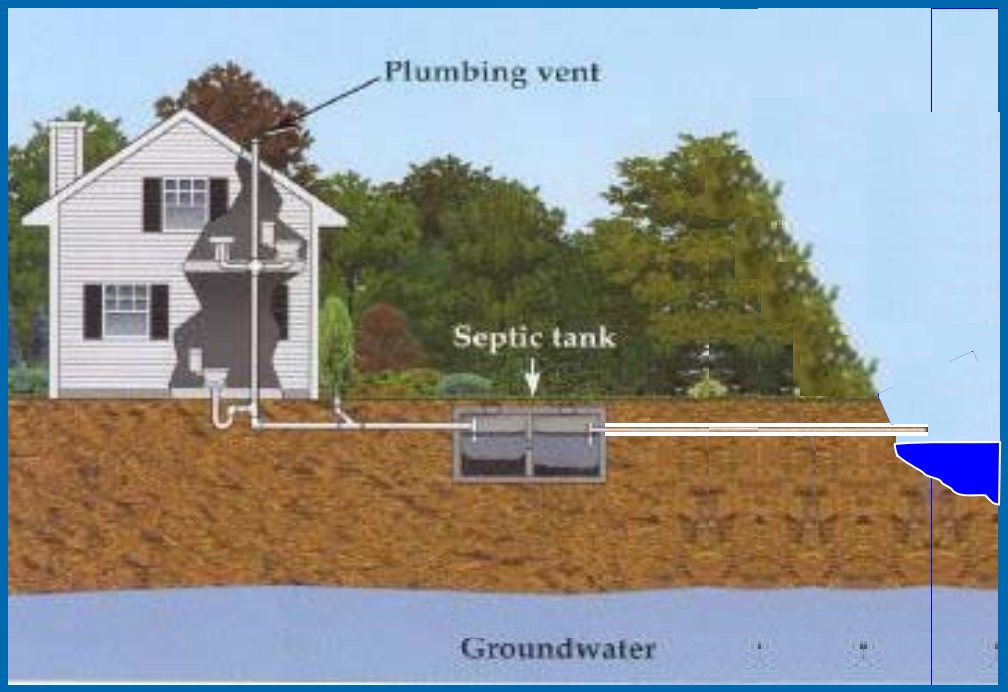
Slide 10

BS4 Should we spread the love and show some pigs too?
BSoenen, 11/16/2010

Sources and pathways



Adapted from: www.thenaturalhome.com

Minnesota Pollution Control Agency

Wisconsin's Water Action Volunteers

Washington State Department of Ecology,
King County and the cities of Bellevue, Seattle and Tacoma

BS3

Sources and pathways

NRCS Photo

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Slide 12

BS3 Do we have any more wildlife pics? Who were the big natural contibutors in Eric's BST studies?
BSoenen, 11/16/2010

Bacteria Source Tracking

- The presence of *E. coli* bacteria in a water sample does not identify the source(s) *(human versus specific type of animal)*
- Bacteria source tracking methods
  - DNA ribotyping
  - Multiple antibiotic resistance
  - Optical brighteners
  - Caffeine and cholesterol (not as effective as once thought)
  - F-Specific Coliphages
  - Sorbitol-fermenting Bifidobacterium

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What influences bacteria levels

- Weather *(levels often increase after rain)*
- Availability of bacteria sources
- Time of Year *(different sources apparent different times of year; i.e., septic systems vs. nonpoint sources)*
- Nutrient levels *(higher levels provide food sources)*
- Sediment *(bacteria can survive a long time in bottom sediments; bacteria can attach to sediment)*
- Freshwater versus seawater *(bacteria survive longer in freshwater)*
- Water temperature *(survive longer in warmer water)*

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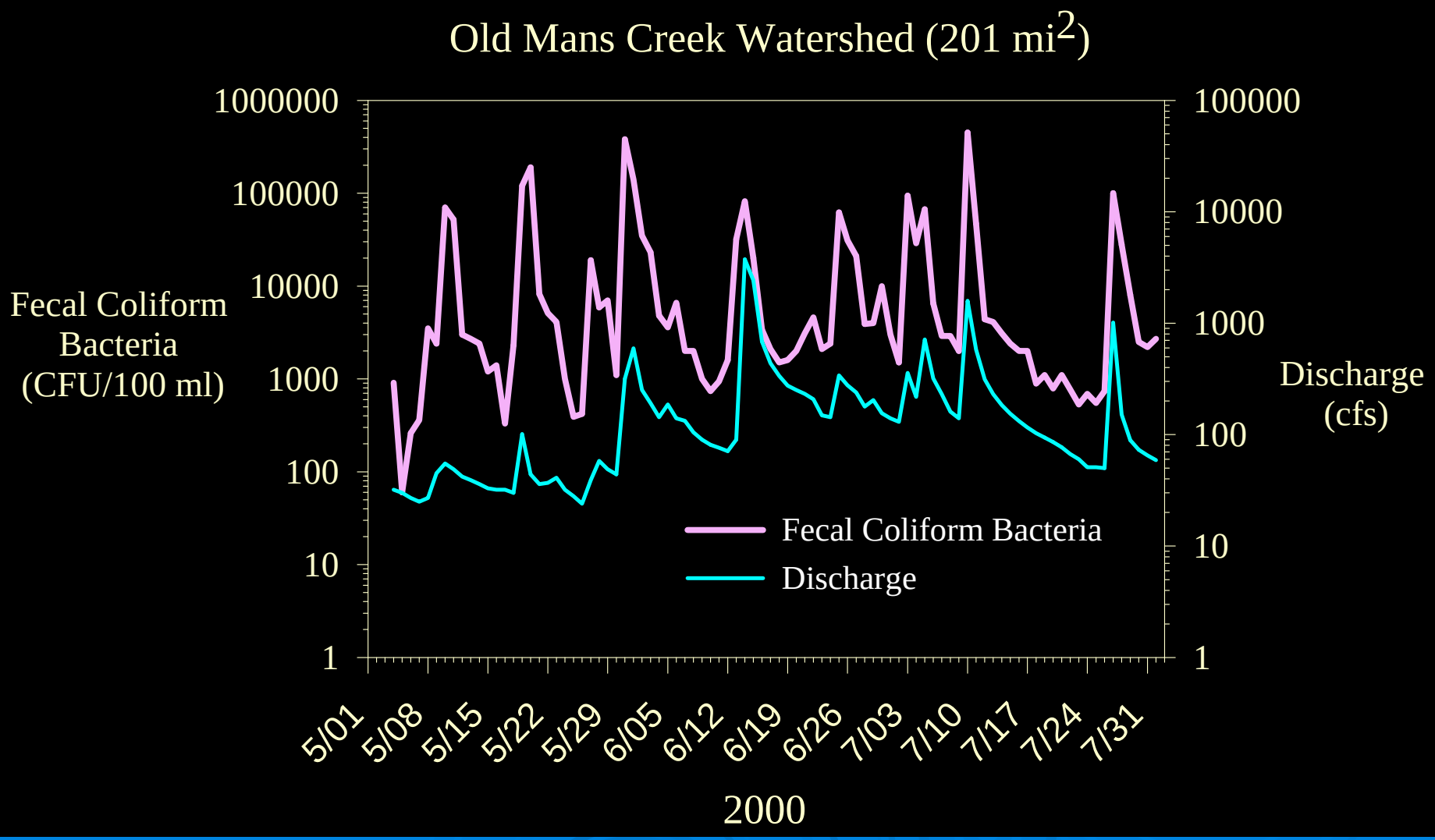
Bacteria levels can be extremely variable



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Human Health Risks

- The U.S. EPA has conducted studies to evaluate swimmer associated illnesses and bacteria levels in waters used by recreational bathers.
- Based on those studies, the U.S. EPA has established bacteria guidelines based on acceptable risk levels to human health.
- At these levels, 8 out of 1,000 swimmers who come into contact with the water may become sick *(more on Iowa's bacteria standards later).*
- As *E. coli* bacteria levels increase, so does the chance that someone will get sick.

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IOWATER Bacteria Monitoring Procedures

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Monitoring Procedures
Preparation

- Avoid contamination of all sampling equipment (inside of Petri dish and tip of eyedropper especially)
- Set-up incubator
  - Use extension cord and night light in Styrofoam cooler provided
  - Put in location where ambient room temperature remains constant

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Monitoring Procedures
Preparation

- Check expiration dates on Coliscan bottles – petri dishes don't expire if stored correctly (room temperature and ***keep bag sealed***)

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Monitoring Procedures
Preparation

Expiration dates on media bottles and boxes

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Monitoring Procedures
Sample Collection

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IOWATER Bacteria Monitoring

Date _____ **Time** _____

IOWATER Monitor _____**Site Number**_____

Other Volunteers Involved

_____

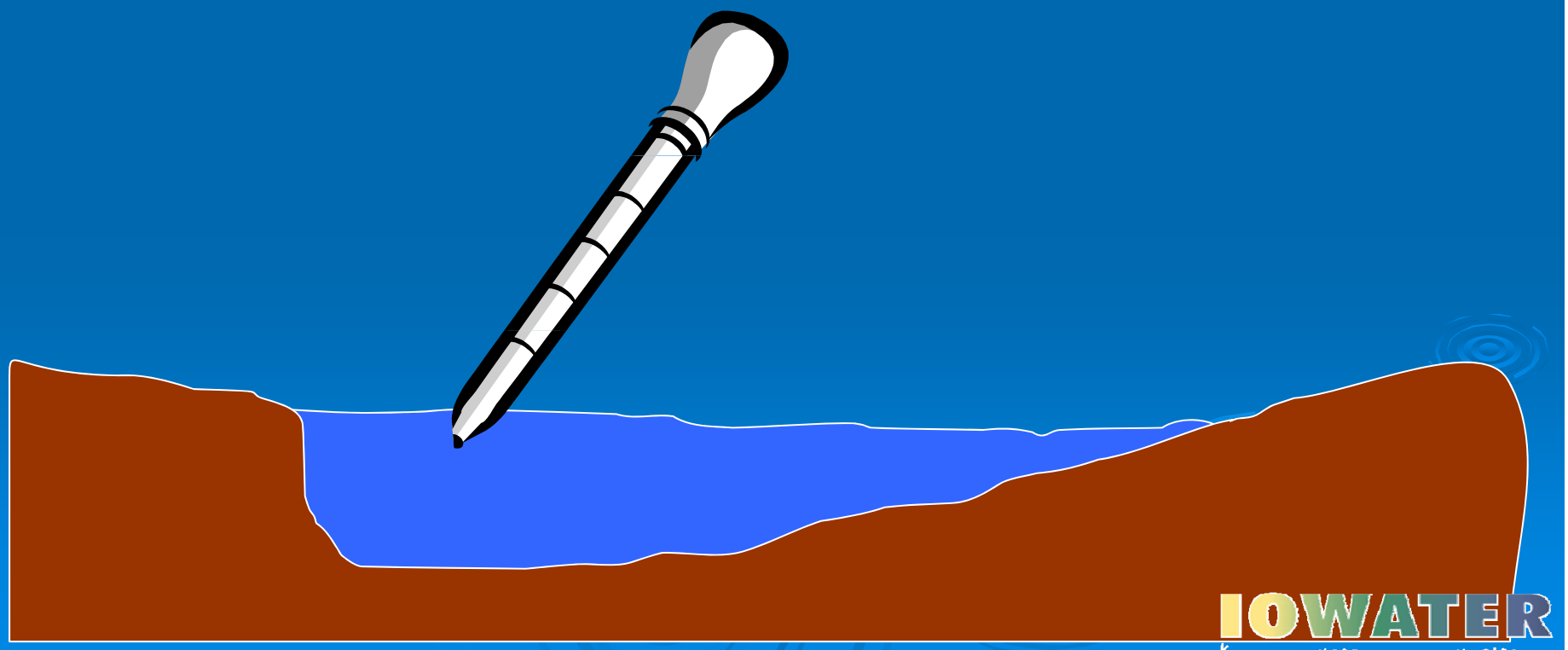
_____

# of Adults (incl. yourself) _____ **# of Children** _____

Note: It is recommended that a Level 1 Chemical/Physical Stream Assessment form

Monitoring Procedures

Sample Collection - Stream



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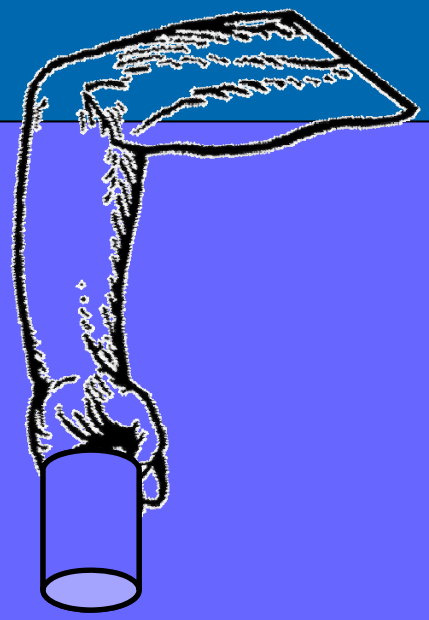
Monitoring Procedures

Sample Collection – Lakes

(remember point sampling)



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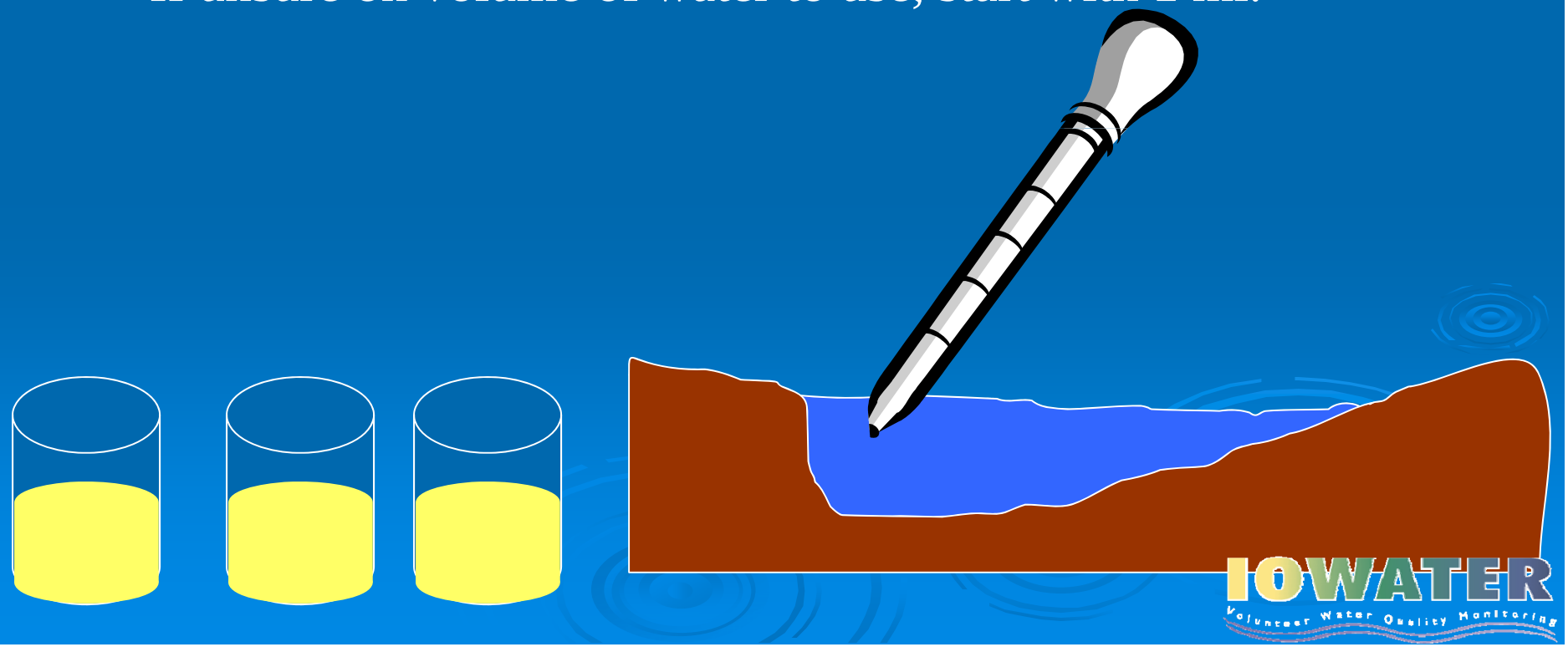
Monitoring Procedures
Sample Collection

- Place sample from pipette directly into Coliscan media bottle
- Repeat procedure 2 times with same pipette
  - Use same volume for each sample!
  - If unsure on volume of water to use, start with 1 ml.



Monitoring Procedures
Sample Collection

- Pack on ice
  - Keep bottles separated from ice
  - Do not use freezer packs

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Exception to sample collection

- If unable to prepare samples for incubation when returning home, a water sample can be stored for up to 24 hours.
- You **must** collect a water sample in a sterile container (collect ~ 50 ml of water) and refrigerate until ready to process your samples.
- Do **not** add the water to the media until you are ready to prepare and incubate your samples.
- When ready to prepare your sample, invert the water sample bottle a couple of times before using a pipette to transfer water to the 3 media bottles.

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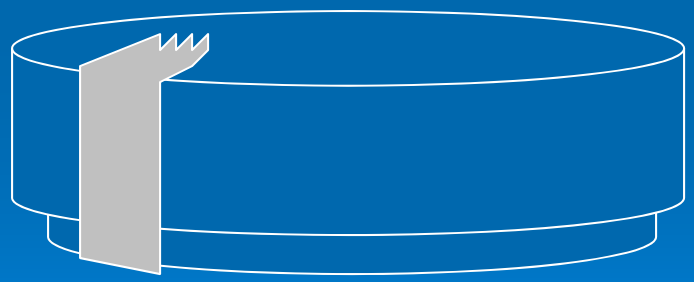
Monitoring Procedures
Preparation



Tape one side of petri dish

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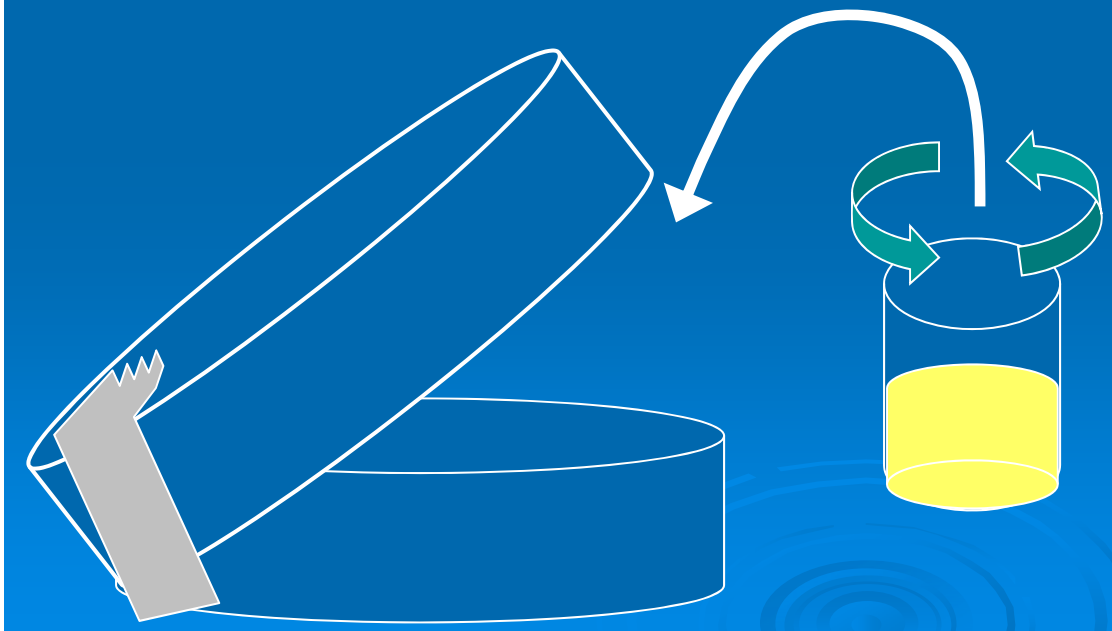
Monitoring Procedures

Preparation

- Label bottom dish
- Label along edges

Monitoring Procedures
Sample Incubation

- Swirl & Pour
- Figure 8 to evenly distribute media
- Allow 45 minutes for medium to solidify – Keep out of sun!



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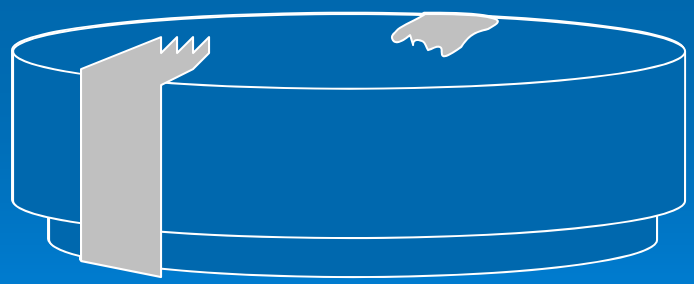
Monitoring Procedures
Sample Incubation

After media is solid

- Tape the other side of the lid
- Turn upside down and place in incubator



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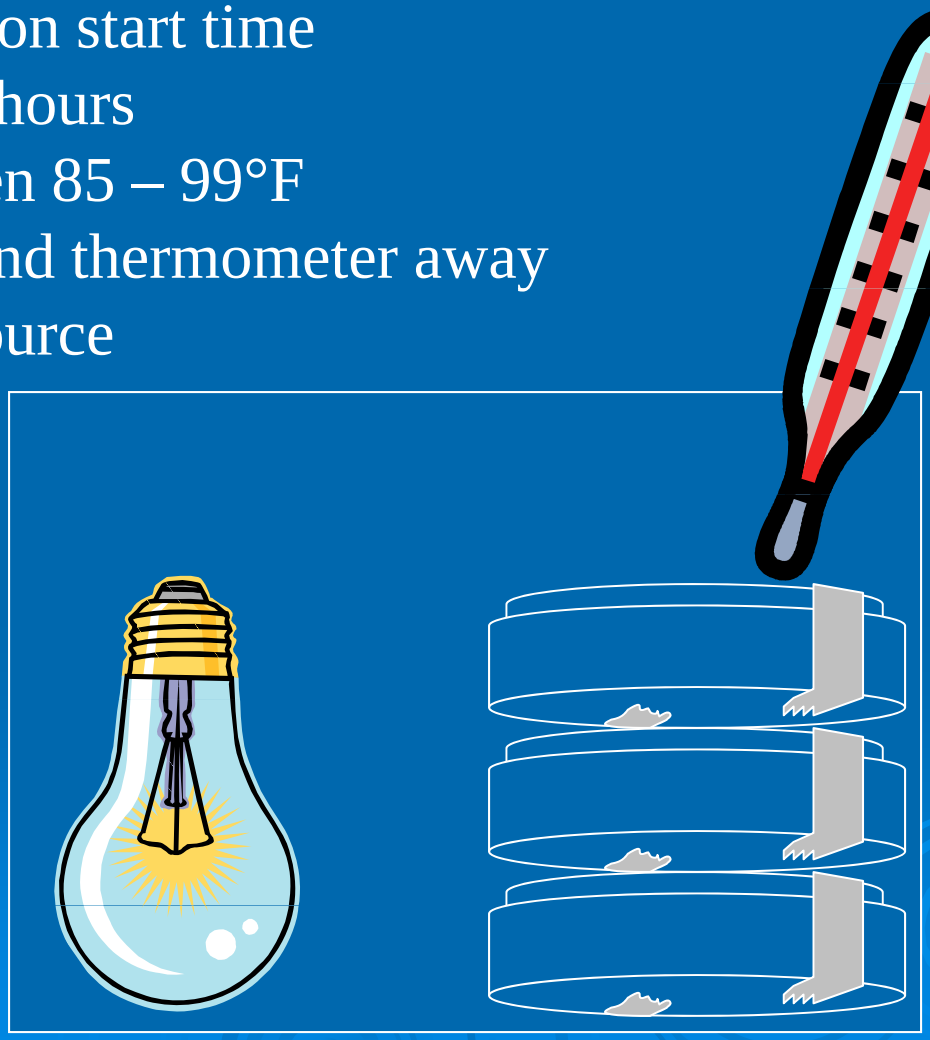
Monitoring Procedures
Sample Incubation

- Record incubation start time
- Incubate for 48 hours
- Incubate between 85 – 99°F
- Place samples and thermometer away from the light source

85 - 99°F



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Monitoring Procedures
Sample Incubation

- Keep the incubation temperature constant
  - Monitor the temperature for the first hour after placing the plates in the incubator
  - If the temperature is too high, open the cooler lid a little
  - If the temperature is too low add another night light (cover the sensor) or cover the cooler with a blanket (watch for fire hazard)

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Monitoring Procedures
Sample Count

Bacteria Numbers	*E. Coli* Bacteria (count) (dark blue-purple)	General Coliform Bacteria (count) (pink)
Replicate 1		
Replicate 2		
Replicate 3		

Volume of Water Sampled for each replicate (ml) _____

Incubation Start Time _____ Incubation End Time _____
Bacteria Incubation Period (hours) _____

Bacteria Incubation Temperature _____ °Fahrenheit

Revised April 2005

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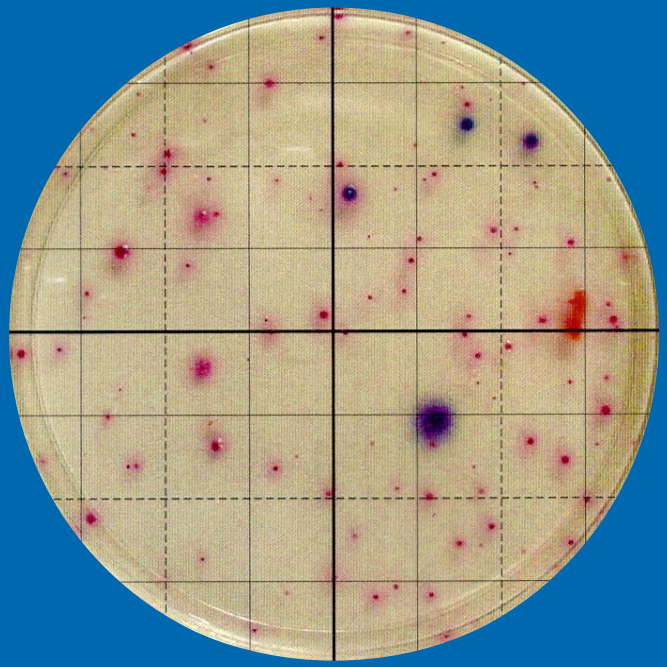
Volunteer Water Quality Monitoring

Monitoring Procedures - Sample Count Coliforms – **Pink** colonies

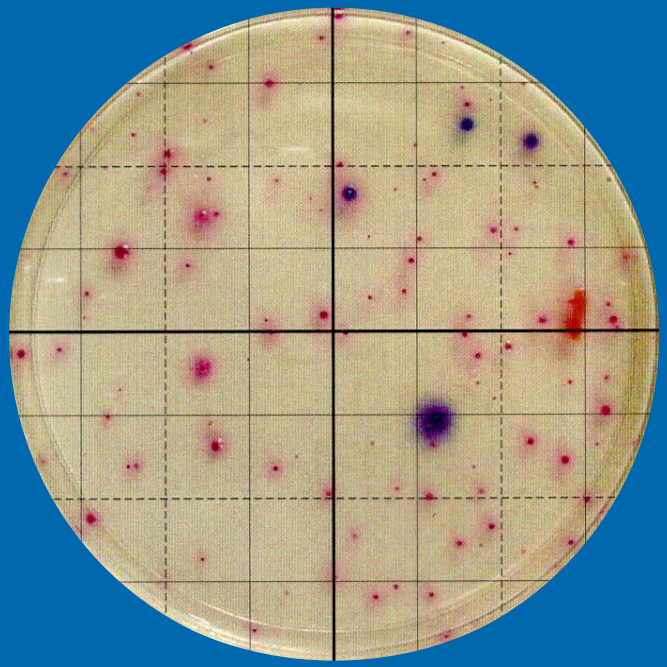
- Use counting grid
- Only count to 100 – “Too numerous to count.”



Presence of ß-galactosidase (from coliform bacteria) turns the colony a pink color

Monitoring Procedures - Sample Count
E. coli – Dark **blue**/**purple** colonies

- Use counting grid
- Only count to 100 – "Too numerous to count."



Presence of ß-galactosidase (produced by coliform bacteria) and ß-glucuronidase (produced only by *E. coli*) turns the *E. coli* colonies a purple (pink + blue) color

What to count as *E. coli*	What not to count as *E. coli*
Purple, no halo	White
Purple with pink halo	Pink, no halo
Purple with purple halo	Pink with pink halo
Blue or dark blue, no halo	Teal green
Blue with purple or pink halo	Pinpoints
Dark blue with teal halo	Teal with teal halo

Monitoring Procedures
Sample Disposal

- Place a teaspoon of bleach in each plate
- Allow it to sit for 5 minutes
- Place bacteria plates in a watertight bag and discard in normal trash
- Allow the cooler to dry before storing

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Stop here to count bacteria on printouts!!!

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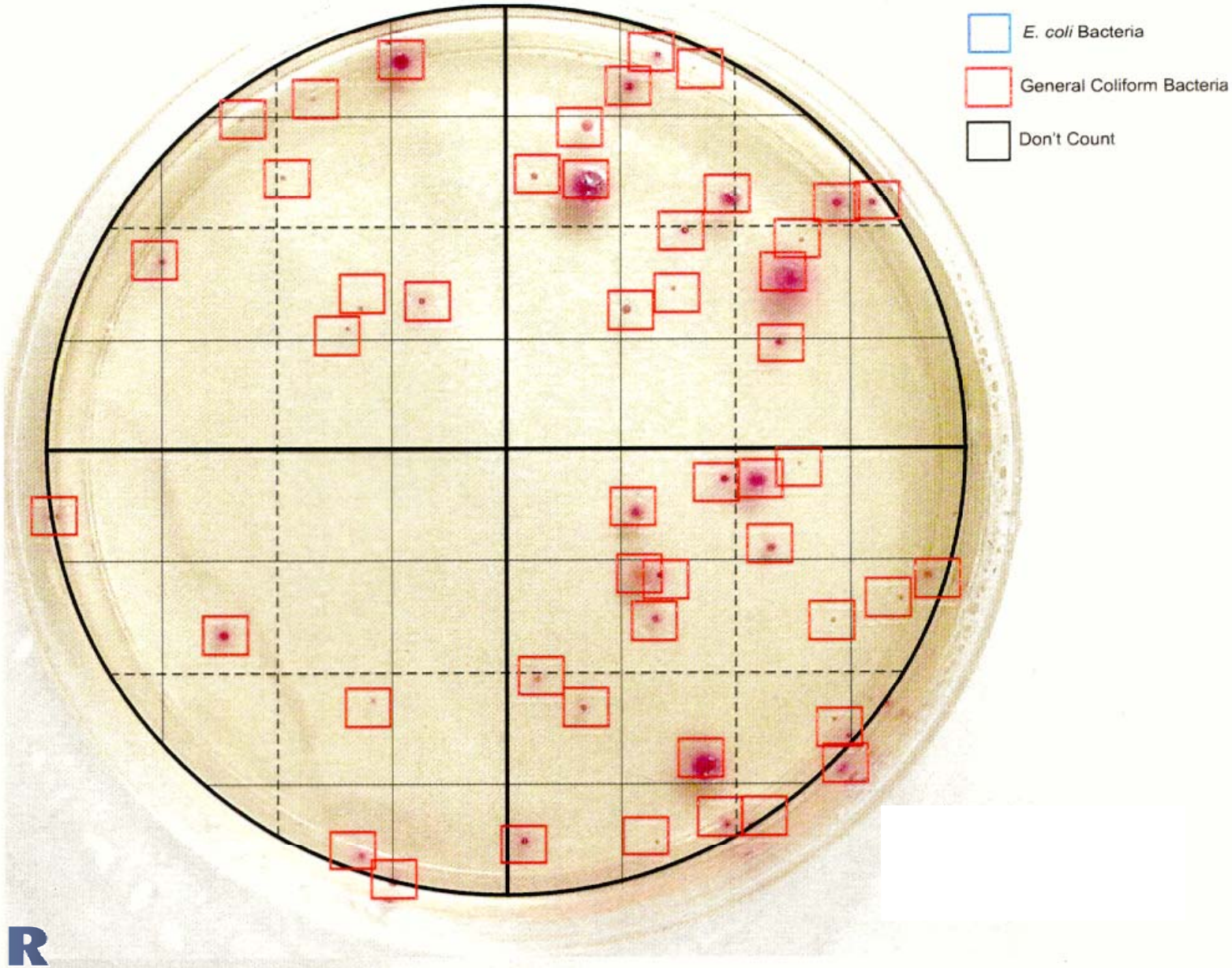
Practice Key

Example 1



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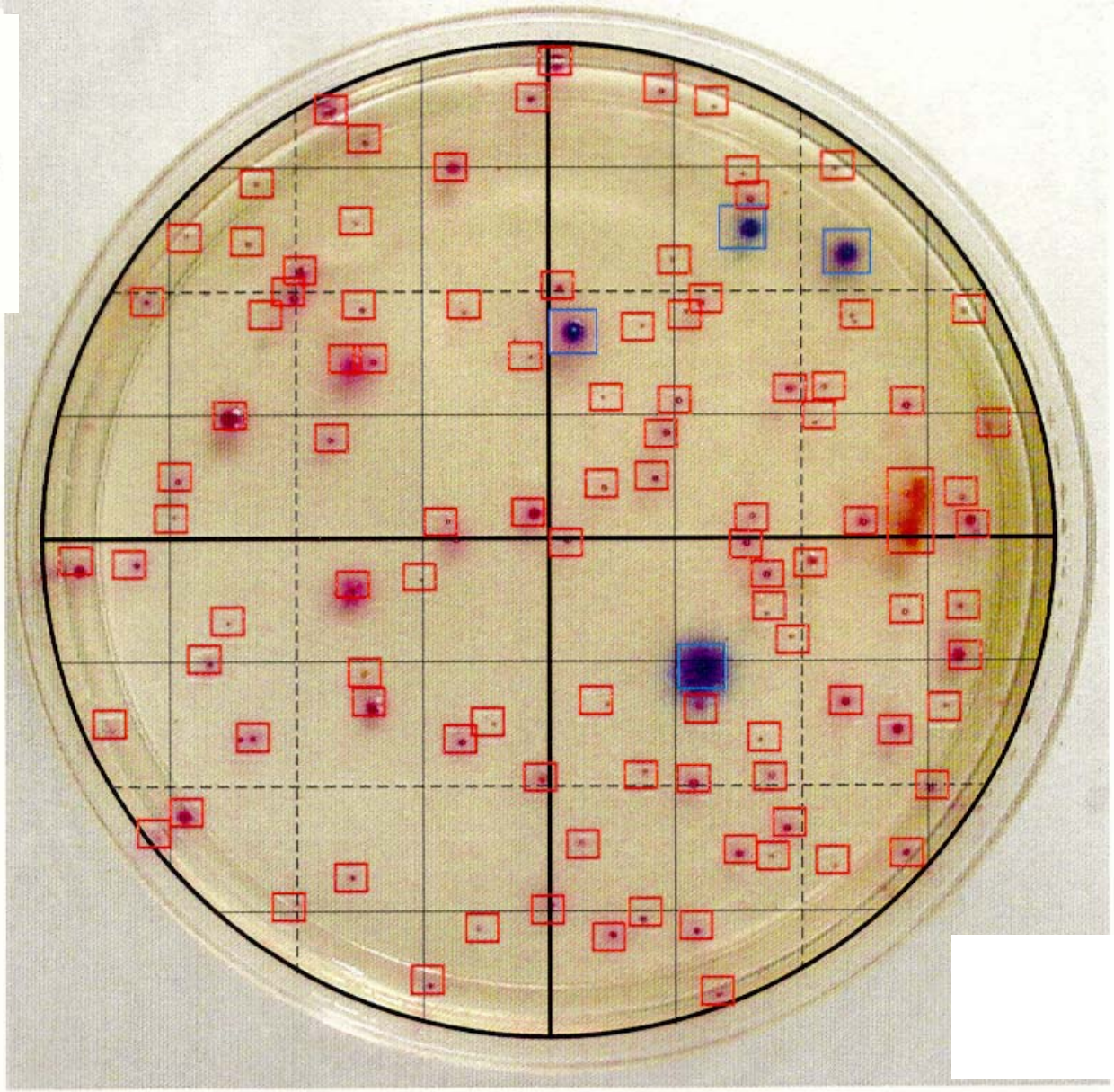
Practice Key

E. coli Bacteria

General Coliform Bacteria

Don't Count

Example 2



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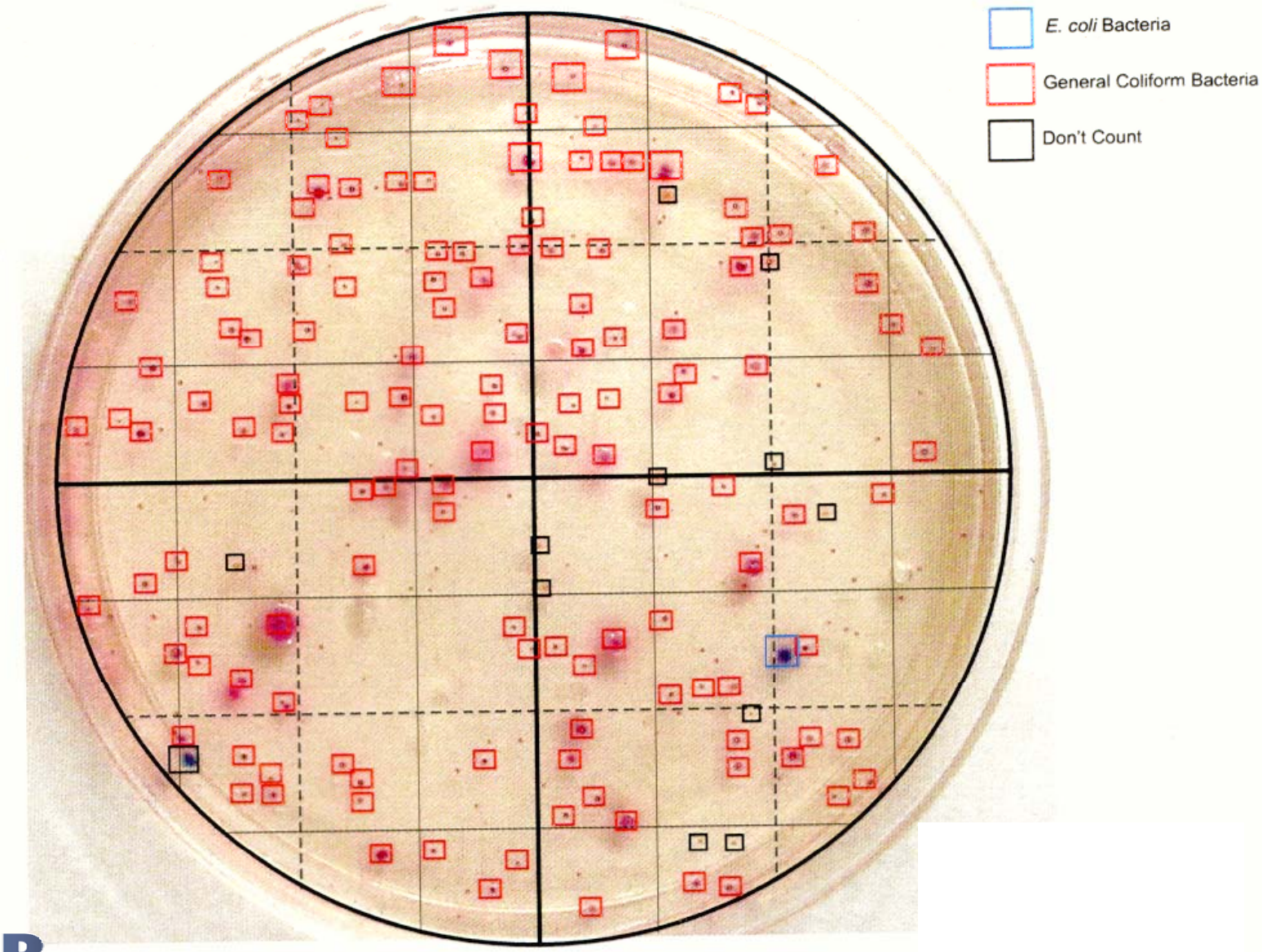
BS5

Practice Key

Example 3



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Slide 41

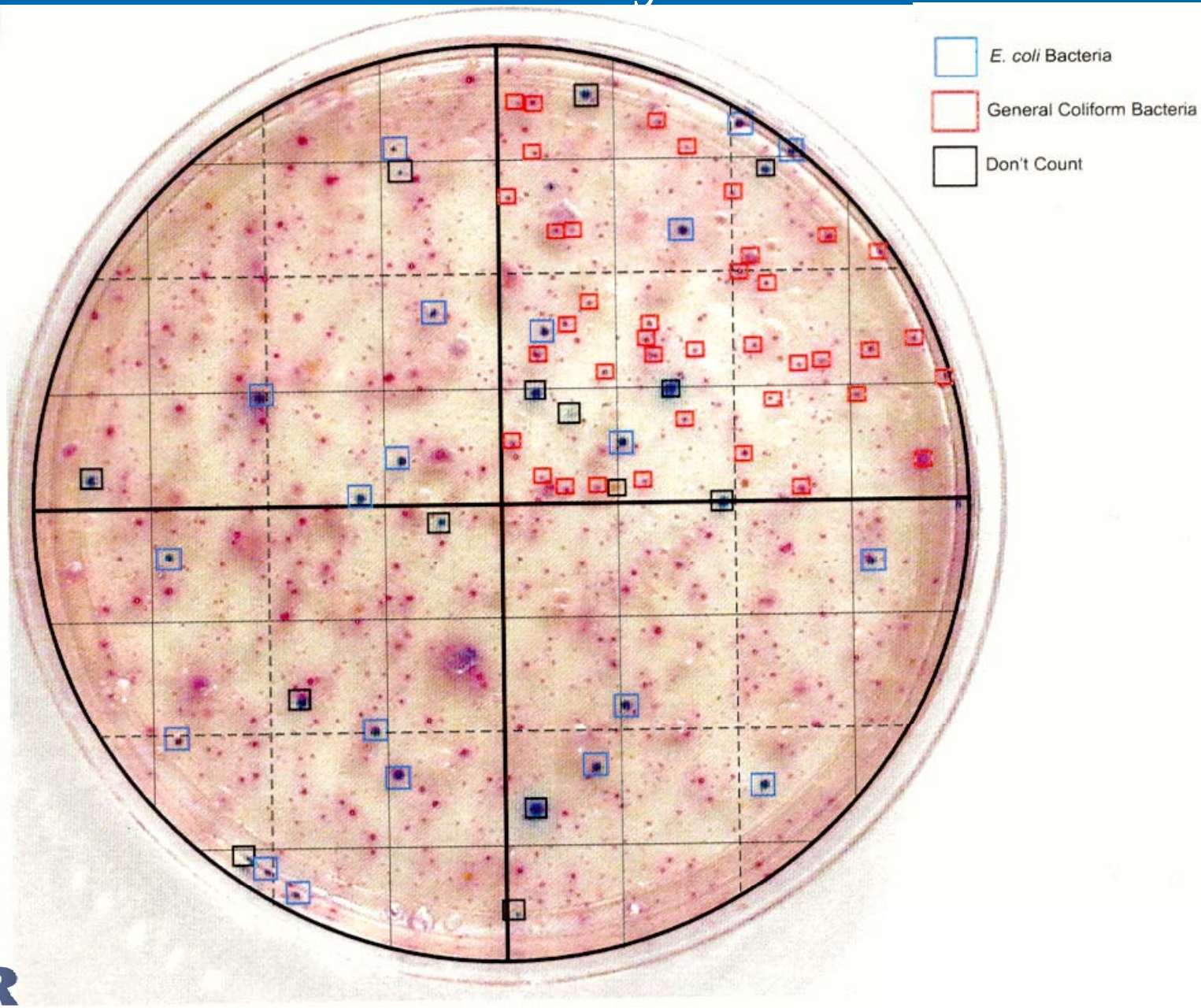
BS5 Shouldn't the "Don't Count" colony in the lower left-hand quadrant be counted as E. coli? It looks to me like it's a navy blue colony with teal halo, something we say should be counted as E. coli.

BSoenen, 11/16/2010

BS6

Practice Key

Example 4



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Slide 42

BS6 It looks to me like all of the "Don't Count" colonies could/should be counted as E. coli. Since this is for demonstration purposes, I'd say that if they look like E. coli on the screen, we could probably count them...might eliminate a little confusion with volunteers.
BSoenen, 11/16/2010

Stop here to count actual bacteria plates!!!

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IOWATER Home - Windows Internet Explorer

http://www.iowater.net/

File Edit View Favorites Tools Help Convert Select

IOWATER Home

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Volunteer Water Quality Monitoring

About | Workshops | Database | Current Volunteers | Learning Corner | Publications | Activities | Links | Contact Us

- Iowa Water Monitoring Atlas
- Get UTM Map Coordinates
- Online Database
  - Register Site
  - Submit Data
  - View Data
- STORET
- Precipitation Information

Popular Links: of Words (ROW) Frequently Asked Questions

Volunteer Monit

August 3, 2010

The Volunteer Monitor newsletter facilitates the exchange of ideas, monitoring methods, and practical advice among volunteer environmental monitoring groups across the nation. The latest issue includes information on Monitoring Impacts of New Gas Drilling Technologies - Freshwater Algae Blooms - Displaying Sites and Data with Google "My Maps" - One-Day Snapshot Finds Hotspots. Click the link below to go directly to the PDF: http://www.epa.gov/owow/monitoring/volunteer/newsletter/volmon21no1.pdf.

For a list of the Internet-available issues, including back issues visit: http://www.epa.gov/owow/monitoring/volunteer/issues.htm.

For information on how to subscribe to this bi-annual newsletter for free visit: http://www.epa.gov/owow/monitoring/volunteer/info.html.

IOWATER Bacteria & Biological Monitoring Workshops Scheduled for this summer

June 21, 2010

The IOWATER Team has scheduled additional workshops for this summer aimed at current IOWATER certified monitors. These workshops include the Bacteria Monitoring Workshop and the Biological Monitoring Workshop. To register please download the registration forms provided on the linked pages above.

IOWATER

Iowater submit data access page - Windows Internet Explorer

http://www.iowater.net/database/submitdata.asp

File Edit View Favorites Tools Help Convert Select

Iowater submit data access page

IOWATER

Volunteer Water Quality Monitoring

Welcome Mark Heuer

Monitoring site selected: 970014 - Mud Creek (Vine Ave - Site 24)

Enter Biological Stream Assessment Data
Enter Chemical/Physical Stream Assessment Data - Level 2
Enter Stream Habitat Assessment Data
Enter Biological Macroinvertebrate Indexing Data
Classify Your Stream
Upload Photographic Records for this Site
Select Another Monitoring Site For Data Entry

To enter bacteria data, select the chemical/physical stream assessment link. Level 2 indicates that you have completed the bacteria workshop.

IOWATER

Chemical/Physical Assessment Data Entry Form - Windows Internet Explorer

http://www.iowater.net/database/Level2/chemphysassessment_entry.asp

File Edit View Favorites Tools Help Convert Select

Chemical/Physical Assessment Data Entry Form

Phosphate as mg/L (Select one) Not Sampled
(dissolved orthophosphate)

Chloride as mg/L Not Sampled
(Select one; if your number is not in the list, check the chloride test strip vial to make sure you converted from Quantab Units to Cl^-)

Water Temperature (degrees Fahrenheit)

Data Entry Page

NOTE: If your bacteria count is >100, enter a value of 100.

Bacteria Counts (from petri dishes)	***E. Coli* Bacteria** (counts)	**General Coliform Bacteria** (counts)
Replicate 1		
Replicate 2		
Replicate 3		

Volume Water Sampled for each Replicate (ml)

Bacteria Incubation Period (hours)

Bacteria Incubation Temperature (degrees Fahrenheit)

Stream Width (meters)

Maximum Stream Depth (along your transect; meters)

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Chemical/Physassessment Data Confirm Page - Windows Internet Explorer

http://www.iowater.net/database/Level2/chemphysassessment_confirm.asp

File Edit View Favorites Tools Help Convert Select

Chemical/Physassessment Data Confirm Page

Chemical	
pH	8
Nitrite-N (mg/L)	0
Nitrate-N (mg/L)	5
Dissolved Oxygen (mg/L)	10
Phosphate (mg/L)	0.1
Chloride (mg/L)	<25

Water Temperature (*65*° F or *18.33*° C)

Confirmation Page

Bacteria Counts	***E. coli* Bacteria**	**General Coliform Bacteria**
Replicate 1 -	4	69
Replicate 2 -	1	73
Replicate 3 -	2	61

Sample Volume (ml) - 1

Incubation Period (hours) - 48

Incubation Temperature (*92*° F or *33.33*° C)

Bacteria (CFU/100 ml) Colony Forming Units/100 ml	***E. coli* Bacteria**	**General General Bacteria**	**Total Coliform Bacteria**
Replicate 1 -	400	6900	7300
Replicate 2 -	100	7300	7400
Replicate 3 -	200	6100	6300

Average *E. coli* Bacteria is ***233*** *CFU/100 ml.*
Average General Coliform Bacteria is ***6767*** *CFU/100 ml.*
Average Total Coliform Bacteria is ***7000*** *CFU/100 ml.*

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Chemical/Physical Log for 970014 - Mud Creek (Vine Ave - Site 24) on June 1, 2010 at 10:00 am - Windows Internet Explorer

http://www.iowater.net/Sitelogs/ChemPhysView.asp?ID=29322&site_no=970014 &datemonitored=6/1/2010&timemonitored=1000

Transparency (Not Sampled centimeters)

pH - 8
Nitrite-N (mg/L) - 0
Nitrate-N (mg/L) - 5
Dissolved Oxygen (mg/L) - 10
Phosphate (mg/L; dissolved orthophosphate) - 0.1
Chloride (mg/L) - <25
Water Temperature 65° F (18.3° C)

Site Log Page

Bacteria Numbers - Colony Forming Units per 100 ml (CFU/100 ml)

	E. Coli Bacteria	General Coliform Bacteria	Total Coliform Bacteria
Replicate 1	400	6900	7300
Replicate 2	100	7300	7400
Replicate 3	200	6100	6300

*The average E. Coli bacteria count was **233** CFU/100 ml.*
*The average General Coliform bacteria count was **6767** CFU/100 ml.*
*The average Total Coliform bacteria count was **7000** CFU/100 ml.*
***1** ml of water was collected.*
*Bacteria samples were incubated for **48** hours at a temperature of **92°F (33.3°C)**.*

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Volunteer Water Quality Monitoring

Water Quality Standards

	Type of Waterbody			
	Rivers/Streams	**Lakes**	**Reservoirs**	**Wetlands**
"General Use" Waters	71,665 miles	92,816 acres	40,850 acres	52,271 acres
"Class A": Primary Contact	2,276 miles 93 segments	44,903 acres 163 lakes	40,850 acres 4 reservoirs	6,296 acres 88 wetlands
"Class B": Aquatic Life	12,186 miles 1,068 segments	47,600 acres 278 lakes	40,850 acres 4 reservoirs	308 acres 1 wetland
"Class C": Drinking Water	286 miles 18 segments	20,350 acres 54 lakes	11,000 acres 1 reservoir	27,273 acres 88 wetlands
"HQ": High Quality	342 miles 50 segments	10,249 acres 7 lakes	none	none
"HQR": High Quality Resource	1,529 miles 109 segments	8,571 acres 5 lakes	11,000 acres 1 reservoir	2,033 acres 5 wetlands

Water Quality Standards

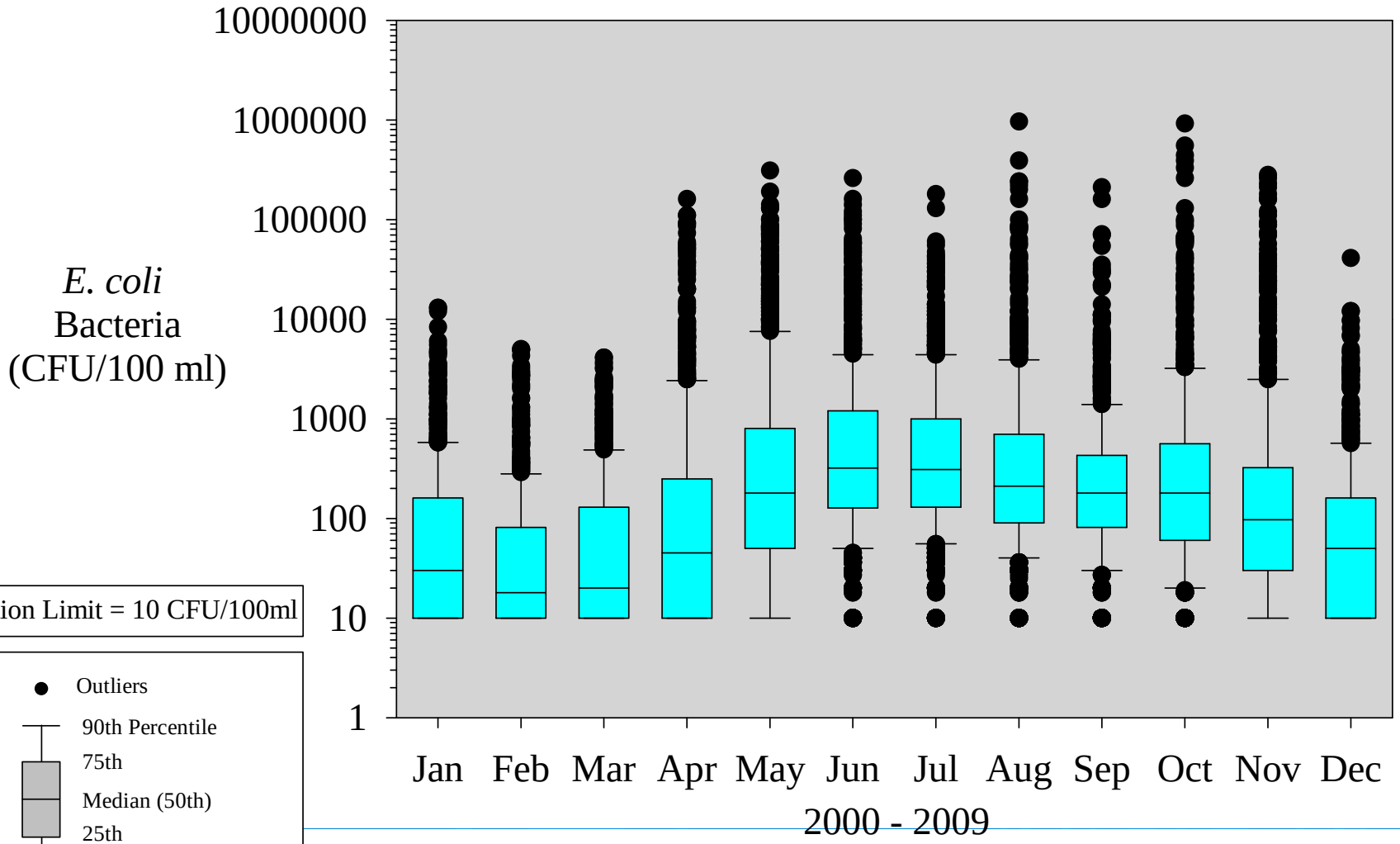
E. coli standards apply to Class A1, A2, and A3 waters

Use	Geometric Mean (CFU/100 ml)	Sample Maximum (CFU/100 ml)
Class A1		
March 15 - November 15	126	235
November 16 - March 14	Does not apply	Does not apply
Class A2 (Only)		
March 15 - November 15	630	2880
November 16 - March 14	Does not apply	Does not apply
Class A2 and B(CW) or HQ		
Year Round	630	2880
Class A3		
March 15 - November 15	126	235
November 16 - March 14	Does not apply	Does not apply

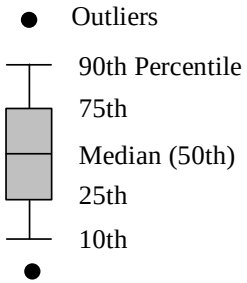
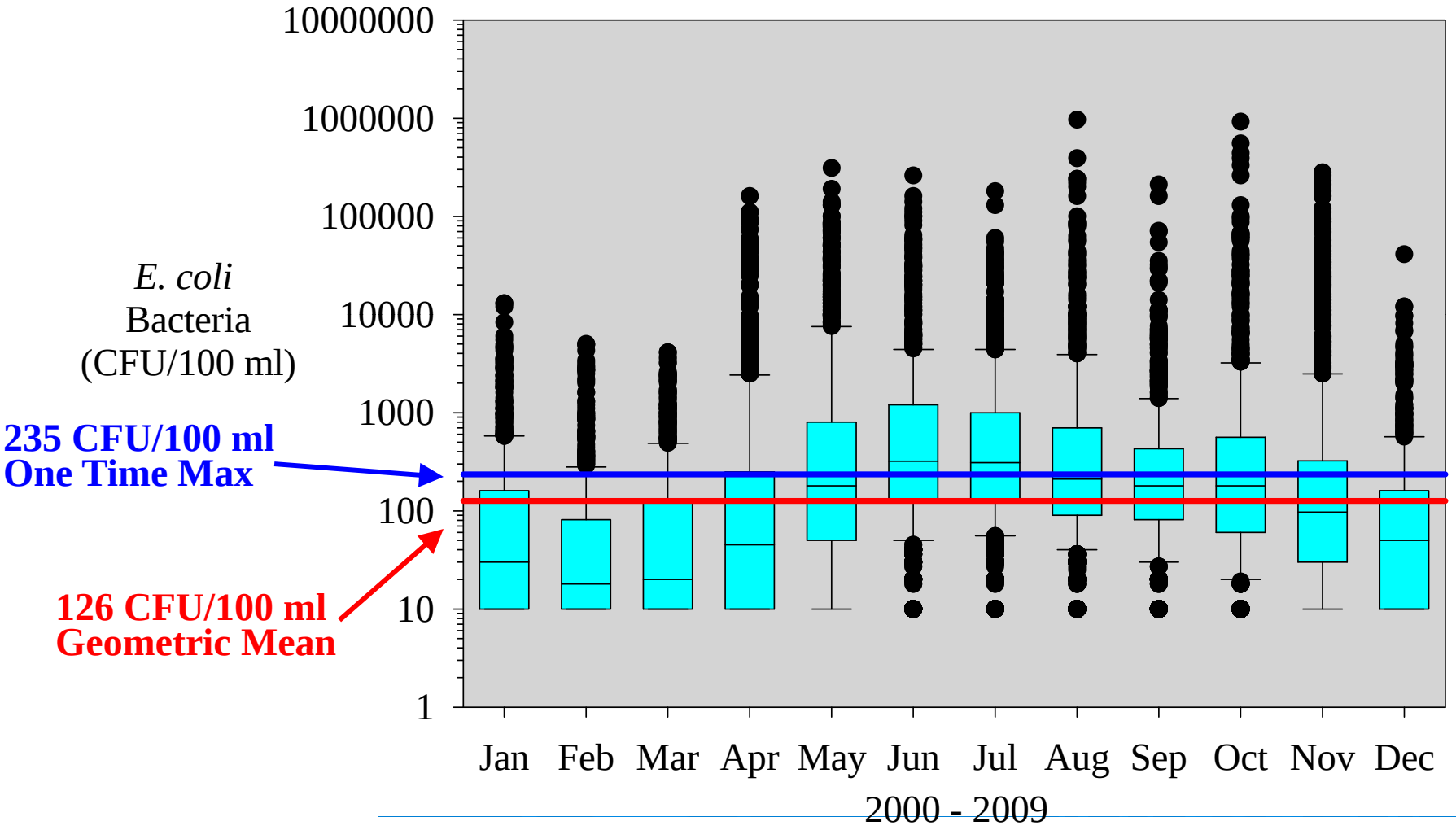
Class A1 – Primary Contact Recreational Use - Swimming
Class A2 - Secondary Contact Recreational Use - Boating
Class A3 – Children's Recreational Use - Wading

IOWATER
Volunteer Water Quality Monitoring

Iowa Ambient Water Monitoring Program - All Stream Sites
E. coli Bacteria (CFU/100 ml) - Monthly Sampling



Quantification Limit = 10 CFU/100ml



Iowa Ambient Water Monitoring Program - All Stream Sites
E. coli Bacteria (CFU/100 ml) - Monthly Sampling