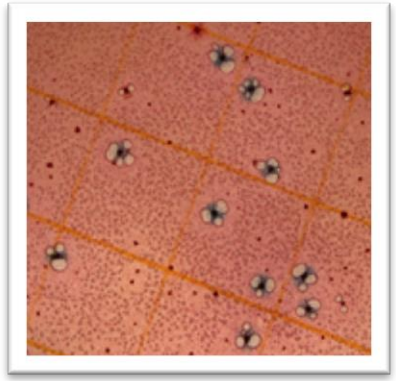
Georgia Adopt-A-Stream
Bacterial Monitoring



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GEORGIA
DEPARTMENT OF NATURAL RESOURCES
ENVIRONMENTAL PROTECTION DIVISION

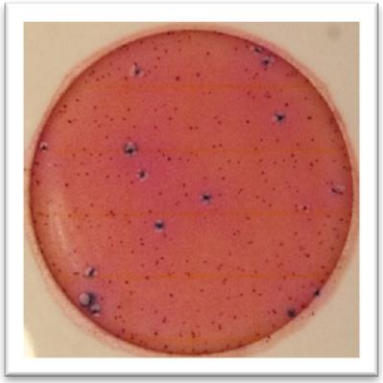
Georgia Adopt-A-Stream

- What is it?
  - Georgia's volunteer water quality monitoring program

- Program Goals
  - **A**: Increase public **awareness**
  - **D**: Collect quality baseline water quality **data**
  - **O**: Gather **observations**
  - **P**: Encourage **partnerships** between citizens & local government
  - **T**: Provide **tools & training**

Bacterial Monitoring

- Involves: collecting, plating, and counting E. coli colonies
- Purpose: **quickly assess health risks due to bacterial contamination of surface waters**
- Characterizes: Stream health by identifying potential for harmful bacteria in stream



EPA Quality Assurance Project Plan

- Quality Assurance/ Quality Control (QA/QC)
- Only individuals are certified
- Certification is valid for one year

- Volunteers must attend an annual recertification workshop
- Only certified volunteers can submit data

To Become a Certified QA/QC Volunteer

FIELD & LAB:

Volunteers must demonstrate how to properly collect and plate a sample

WRITTEN TEST:

Volunteers must pass a written evaluation with a score of at least 80%

&

Volunteers must correctly identify E.coli colonies and calculate E.coli levels of example plates with accuracy of at least 90%

What is a Watershed?

- A watershed is the land area from which water, sediment, and dissolved materials drain to a common point along a stream, wetland, lake or river.

- Its boundaries are marked by the highest points of land around the waterbody.

When, Where, & How Often?

Where: Same site location and in a well mixed area of flowing water

When: Same time of day and during normal flow conditions

How often: At least **once a month**

Safety Considerations

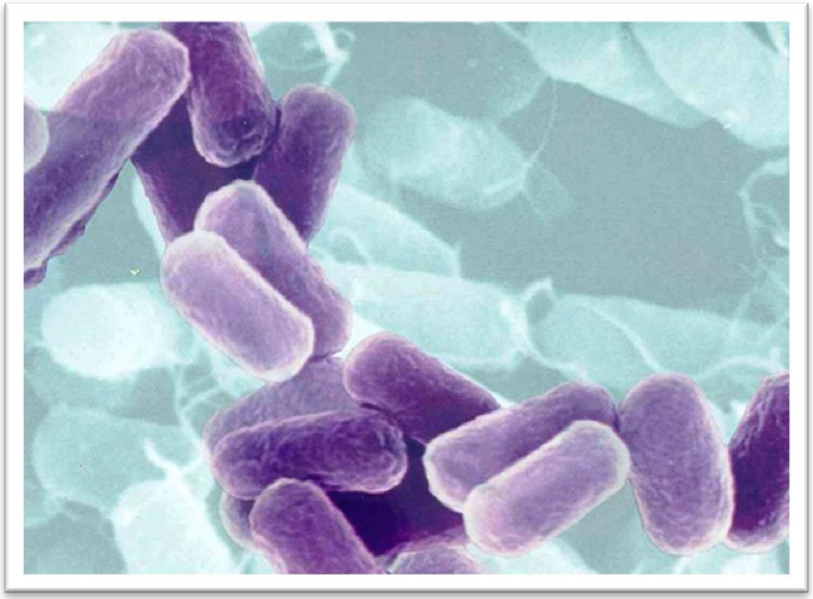
If conditions are too dangerous to sample…

DON'T SAMPLE!

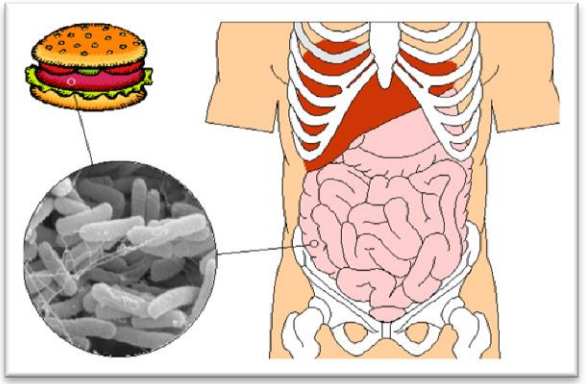
- Wait until storm has stopped and strong flow has subsided
- Never sample alone
- Remember to wear gloves and boots
- Disinfect and dispose of used plates properly
- Receive permission from land owner before going onto private property

What are Bacteria?

- **Single-celled, living microscopic organisms**
- There are more bacteria on Earth than any other living thing
- Found all over the world in many different environments



Why are Bacteria Important?



Benefits

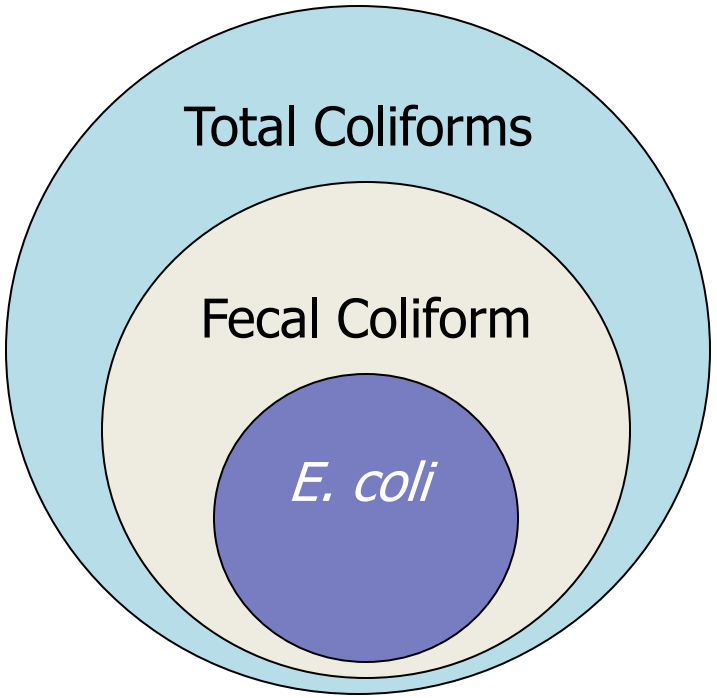
- Decomposition
  - Digestion
- Nutrient cycling
- Can be used to aid in pollution control
  - Sewage treatment
  - Oil spills
  - Other pollutants

Risks

- Some bacteria that live in soil, water or air can cause human, animal and plant health problems
  - Pathogenic bacteria

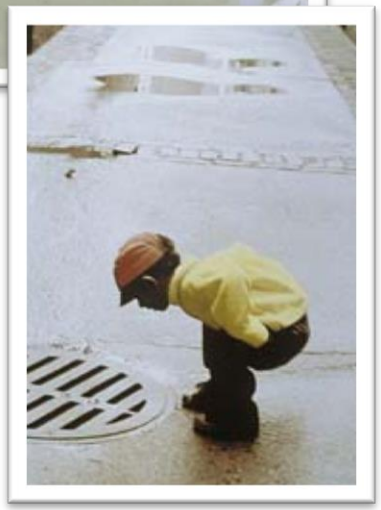
Coliform Bacteria

- Total Coliform refers to a biological family of bacteria that are naturally found in soil
- Fecal coliforms are a subgroup within coliform bacteria found in intestinal tracts of humans and other warm-blooded animals
- E. coli are one subgroup of fecal coliform



Why Monitor for E. coli ?

- E. coli serve as an indicator species
  - **High levels indicate possible presence of pathogens**
- Sources of E. coli in our waterways
  - **Wildlife**
  - **Livestock**
  - **Urban storm runoff**
  - **Leaking pipes**
  - **Failing septic systems**



Weather and Seasonal Influences

- **Bacteria numbers often increase following a heavy rainstorm or heavy runoff event**
- E. coli counts are often higher in summer compared to lower counts in winter months

Warmer surface water temps = higher replication rates for E. coli

Colder surface water temps = lower replication rates for E. coli

6 Steps for E.coli Monitoring

1. Preparing the blank/control
2. Collecting a sample
3. Plating your samples and blank
4. Incubating
5. Counting
6. Disposing

1. Preparing the blank/control

Creating a blank checks to see if sampling methods allow for contamination.

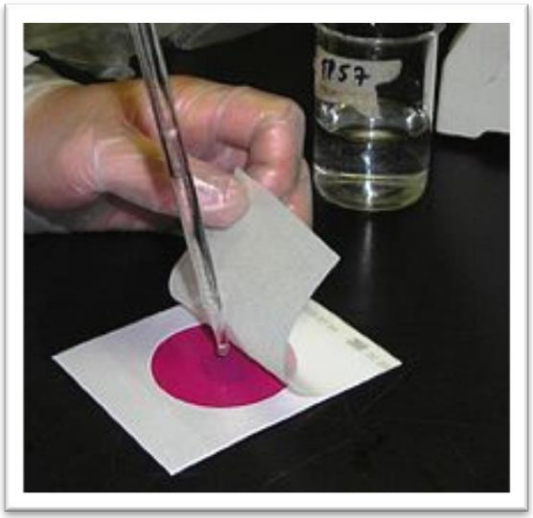
- Label Whirl-pak® bag as a blank
- Wear gloves and remove the perforated seal
- Use small white tabs to pull open the bag
- Fill the bag 2/3 full with distilled water (brought from home)
- Whirl!
- Place blank in a sanitized cooler with ice where other sample will be stored

2. Collecting a sample

- Use one Whirl-pak® to sample upstream of yourself
- Clearly label your sample
- **Place sample in your cooler after collection**
  - **Exposure to UV will decrease bacteria levels**
- **Plate sample as soon as possible, but the maximum holding time on ice is no more than 24 hours**

3. Plating your sample

- Clean work area with disinfectant spray
- Before plating, invert sample to mix the water
- Prepare 3M Petrifilm plates
  - Check expiration dates!
  - Label: Blank, 1, 2, 3 (+ site name)
- **Run in triplicate + 1 blank (3 plates from your one sample + 1 control/blank plate)**
  - **4 plates total**



4. Incubating

- **35°C ± 1 degree for 24 hours ± 1 hour**
  - Turn on early to get stable temperature reading with a digital thermometer inside
  - Adjust using metal dial on top

- Check minimum and maximum temperatures after incubating

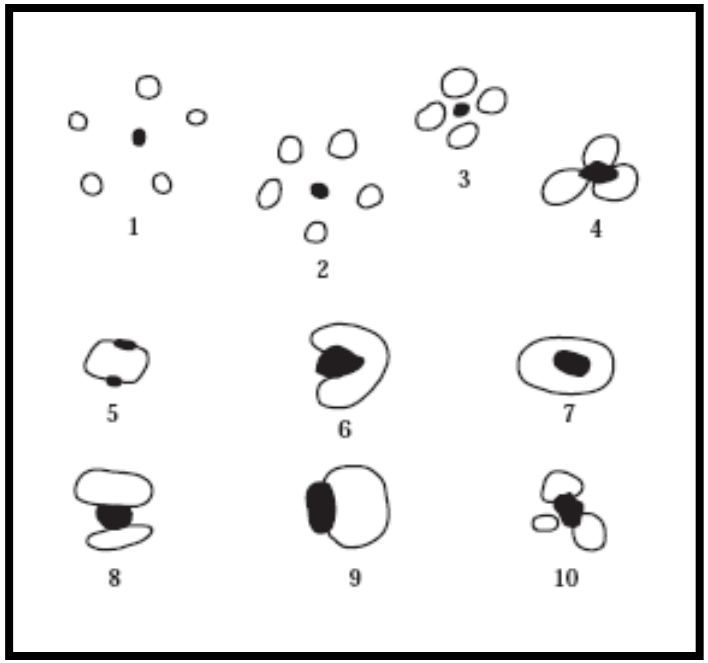
5. Reading the Results…

counting the *E.coli* colonies

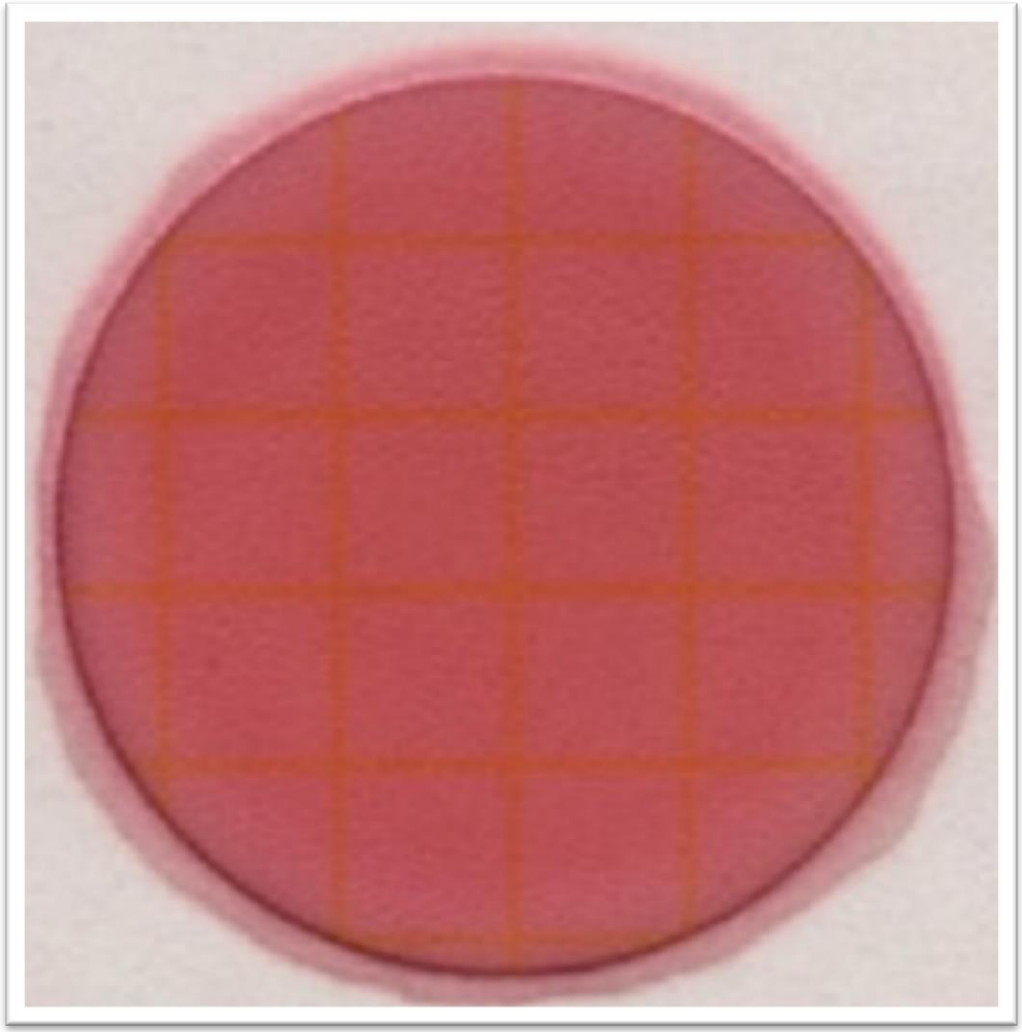
- Standard reporting units for bacteria are in **CFU/100 ml → Colony Forming Units per 100ml**
- Only count **blue colonies with entrapped gas bubbles!**
- Do not count colonies that are growing more than halfway off of the medium

Possible gas bubble patterns associated with gas producing colonies.

All of these examples would be counted when reading plates.



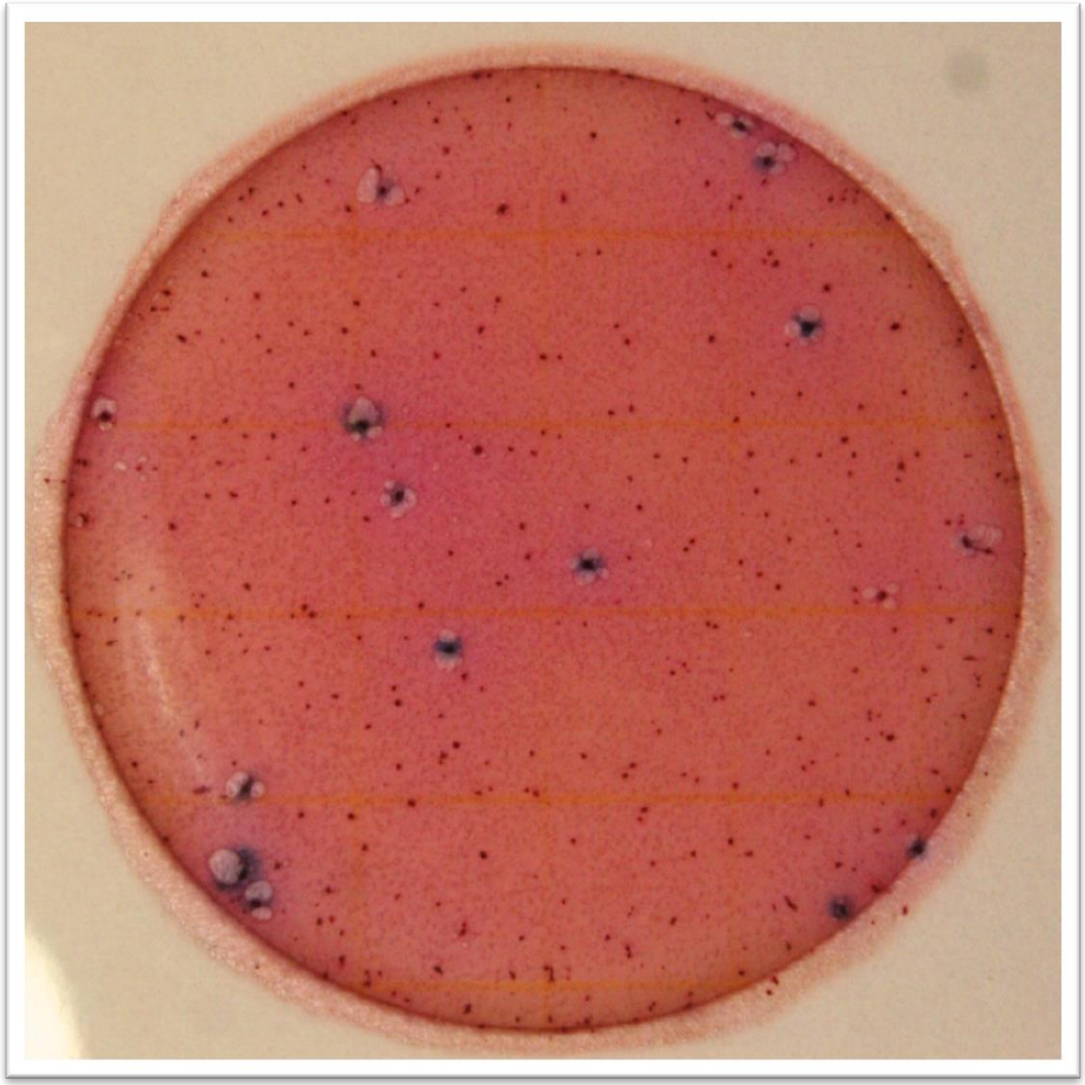
The Blank/Control



There should not be any colonies on the blank.

If any colonies appear on blank, sample is null and void! And new sample must be taken from site location.

Example #1

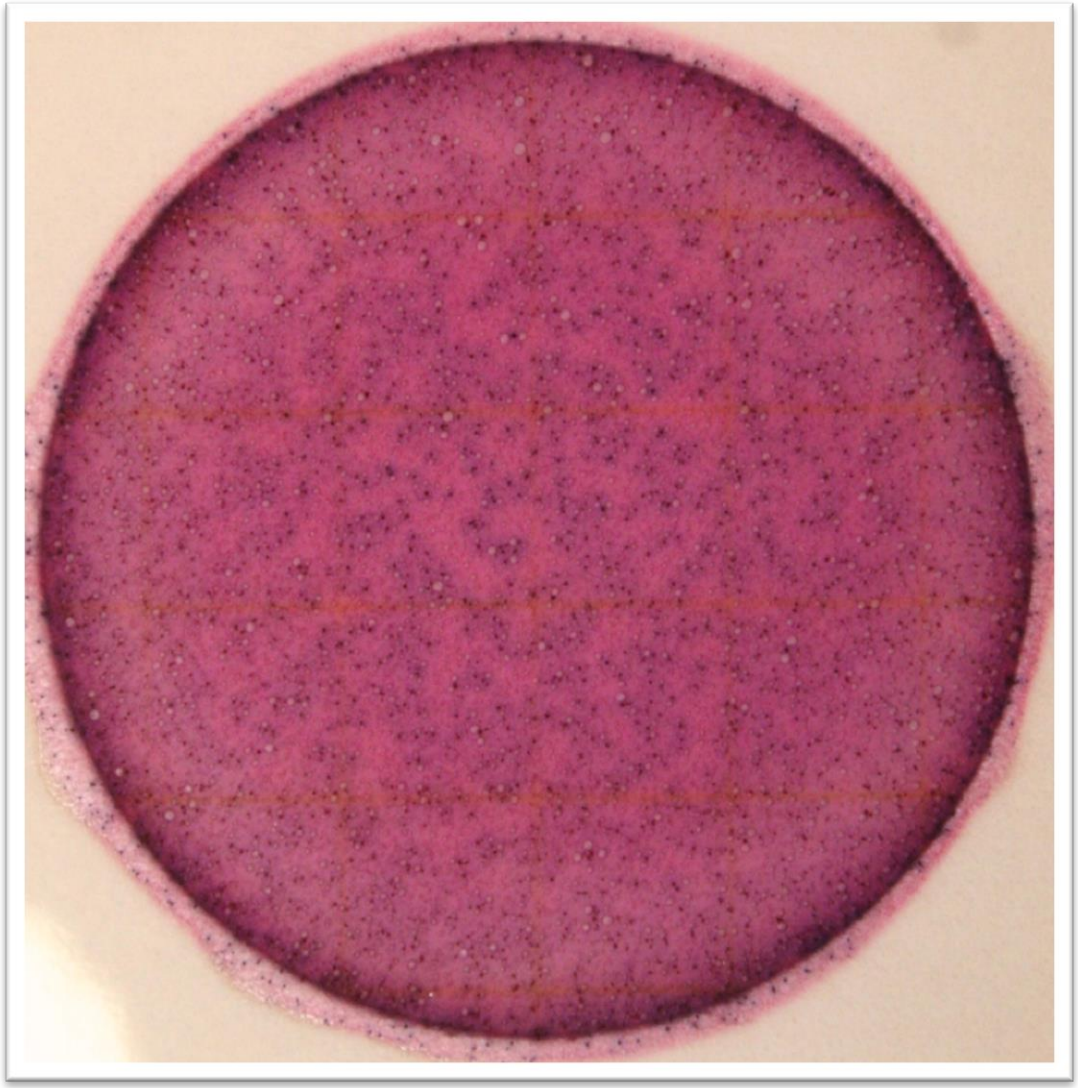


How many *E. coli* colonies can you see?

Example #2

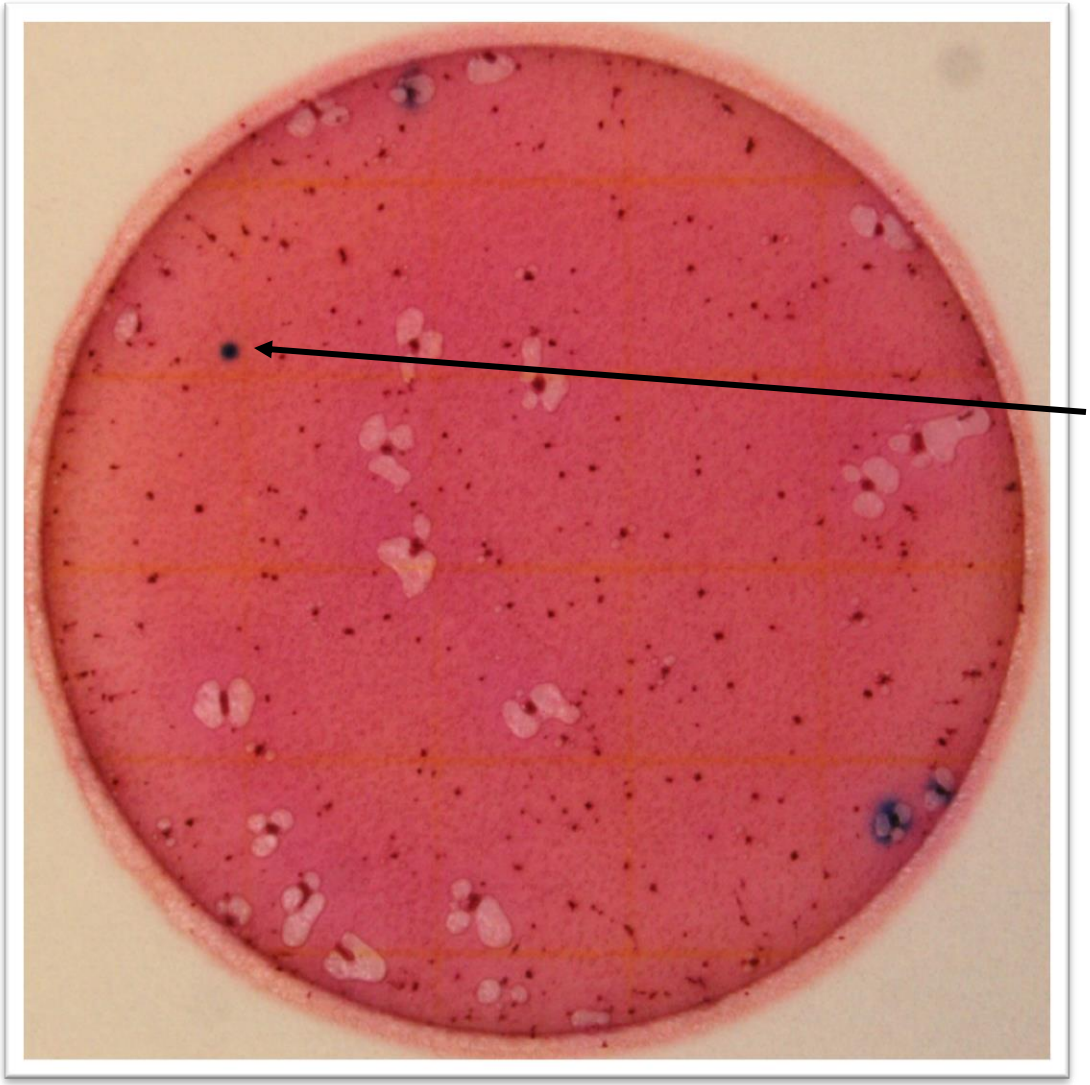
How many *E. coli* colonies can you see?

Example #3



How many *E. coli* colonies can you see?

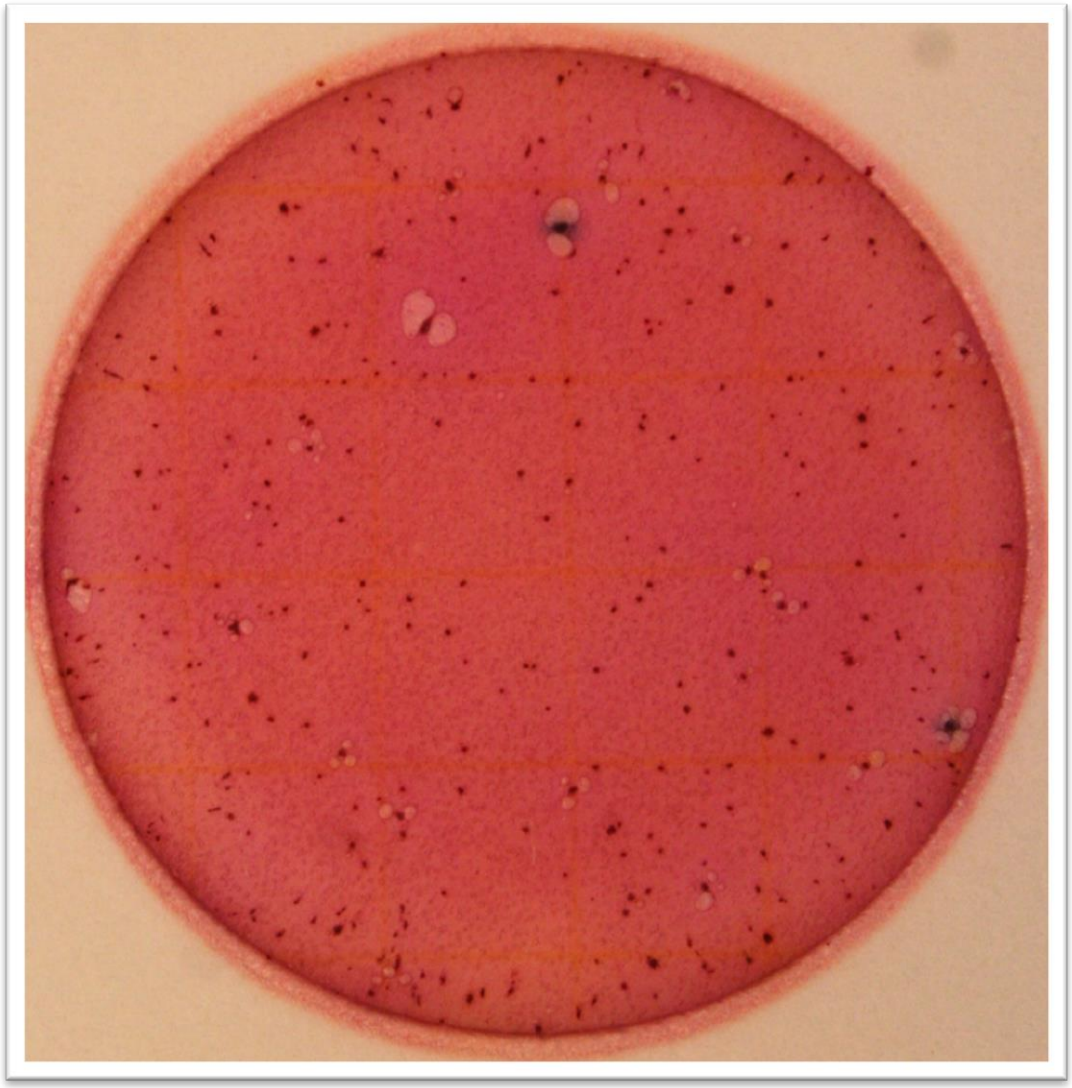
Example #4



How many *E. coli* colonies can you see?

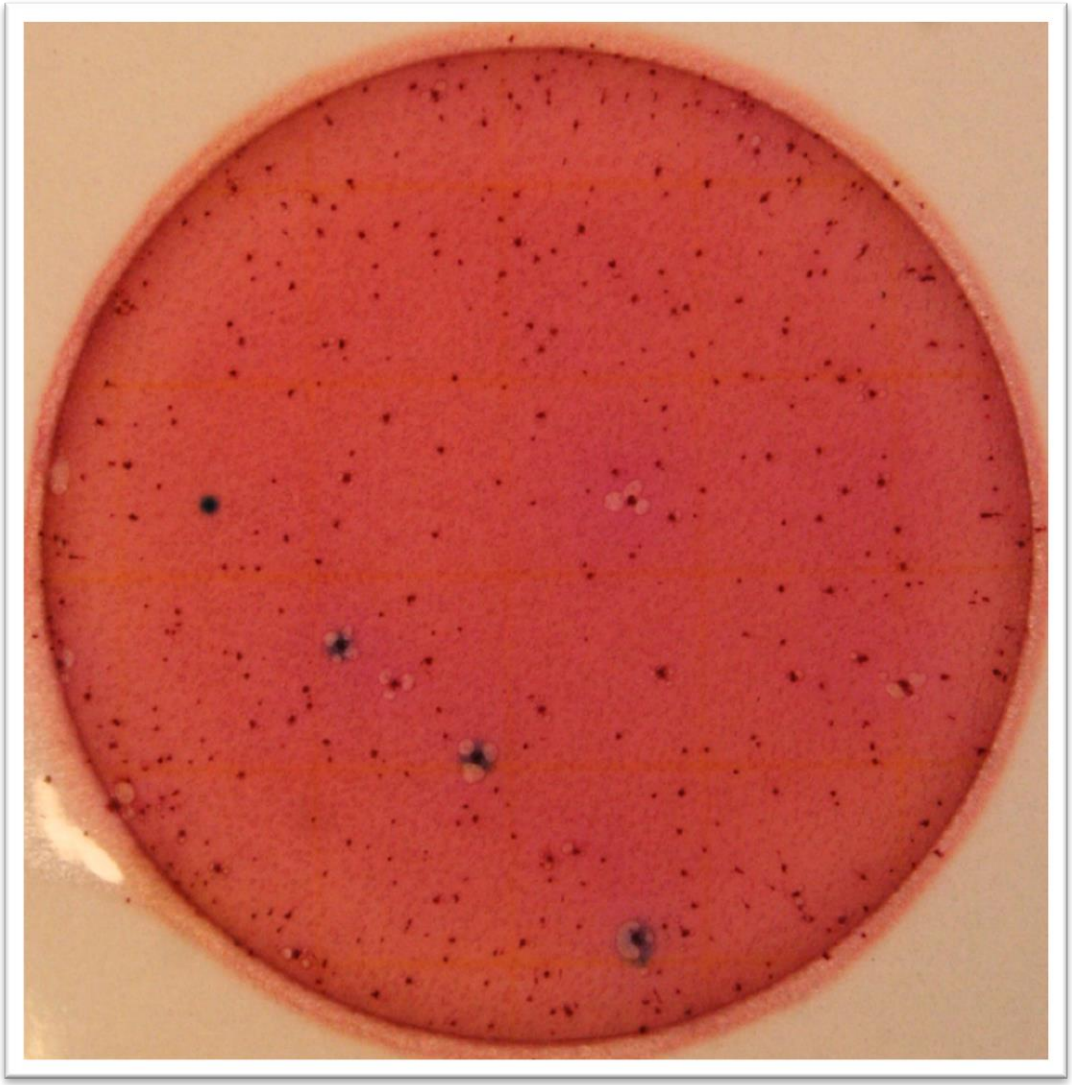
Do not count blue colonies without gas bubbles

Example #5



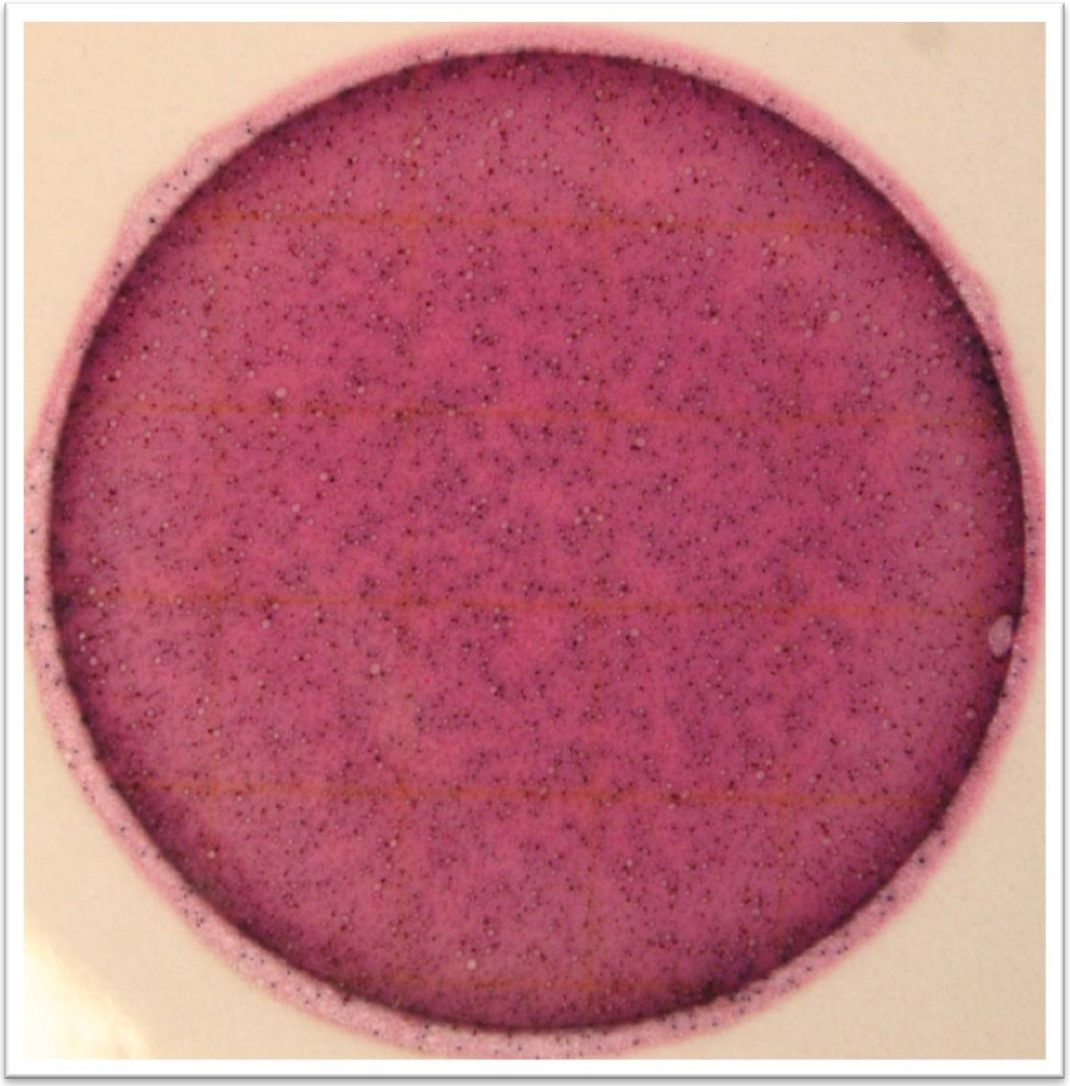
How many *E. coli* colonies can you see?

Example #6



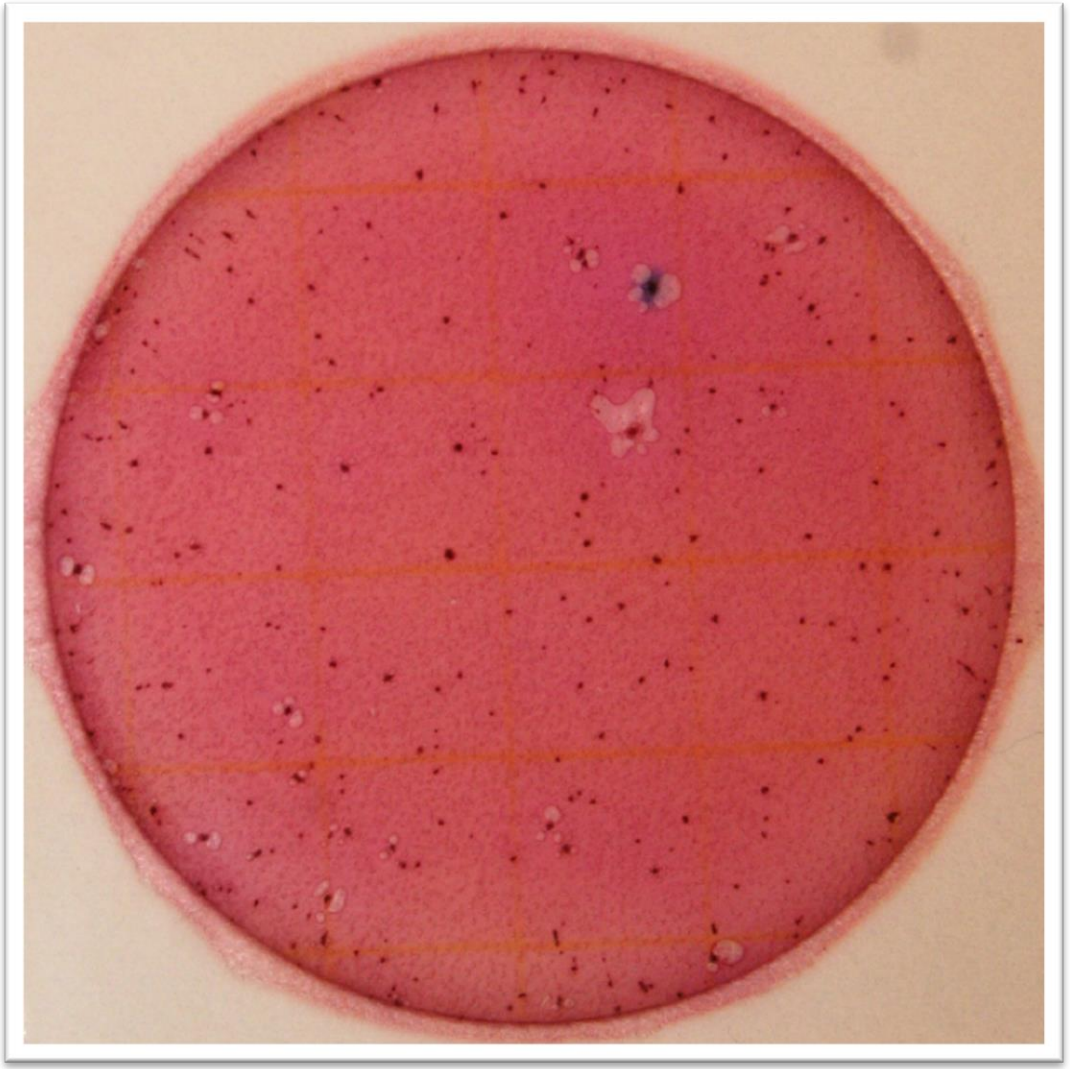
How many *E. coli* colonies can you see?

Example #7



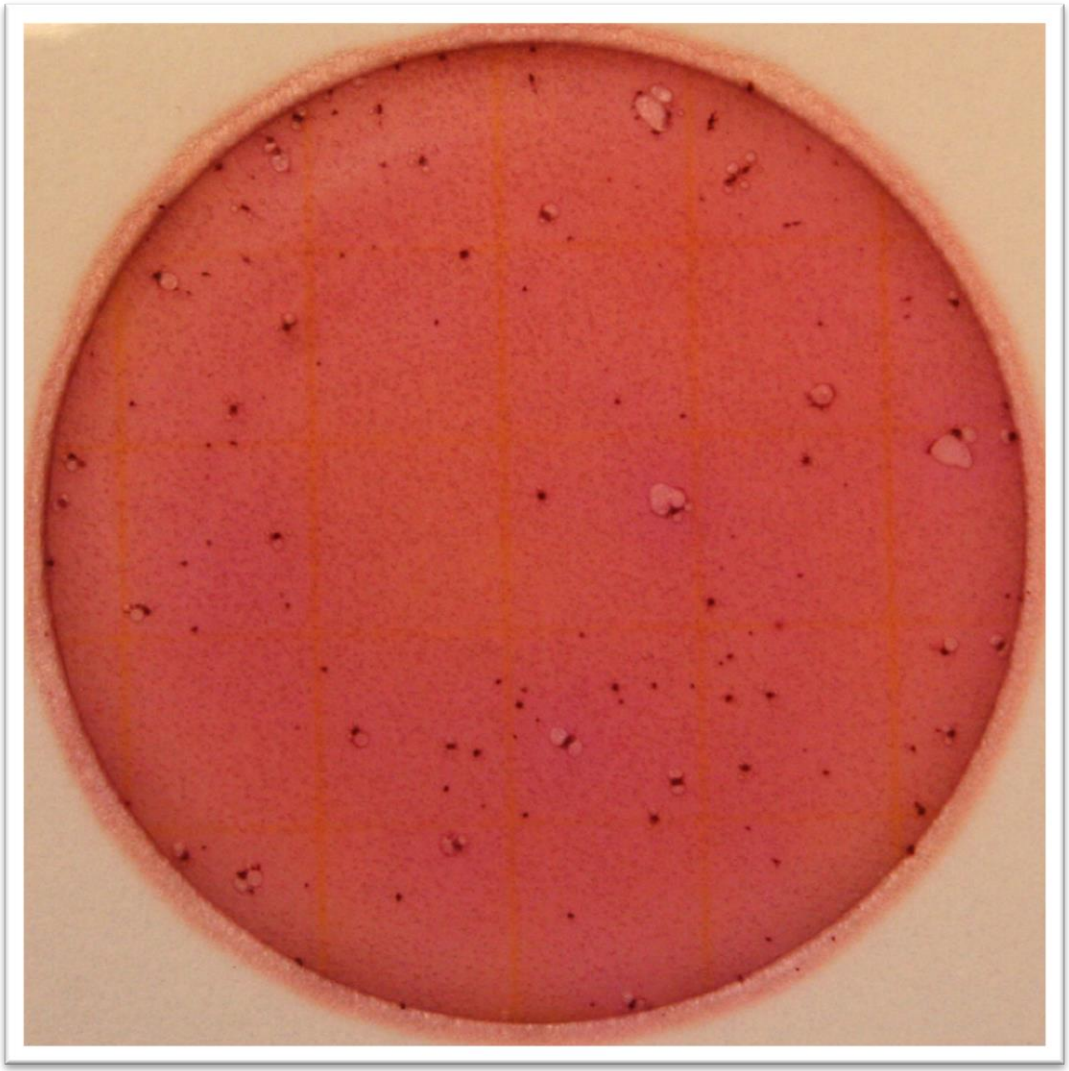
How many *E. coli* colonies can you see?

Example #8



How many *E. coli* colonies can you see?

Example #9



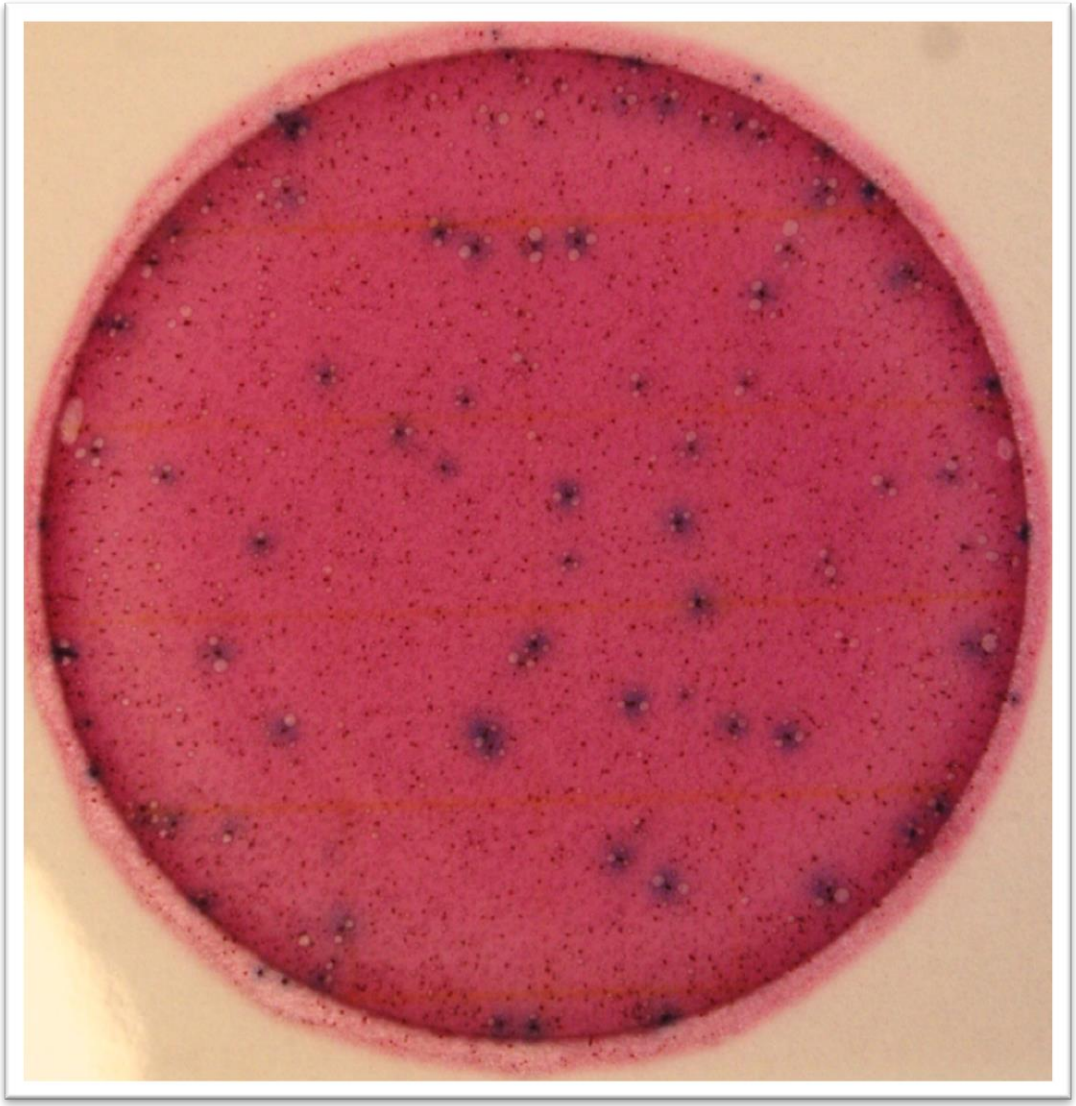
How many *E. coli* colonies can you see?

Would this plate be acceptable as a blank?

Example #10

How many *E. coli* colonies can you see?

Example #11

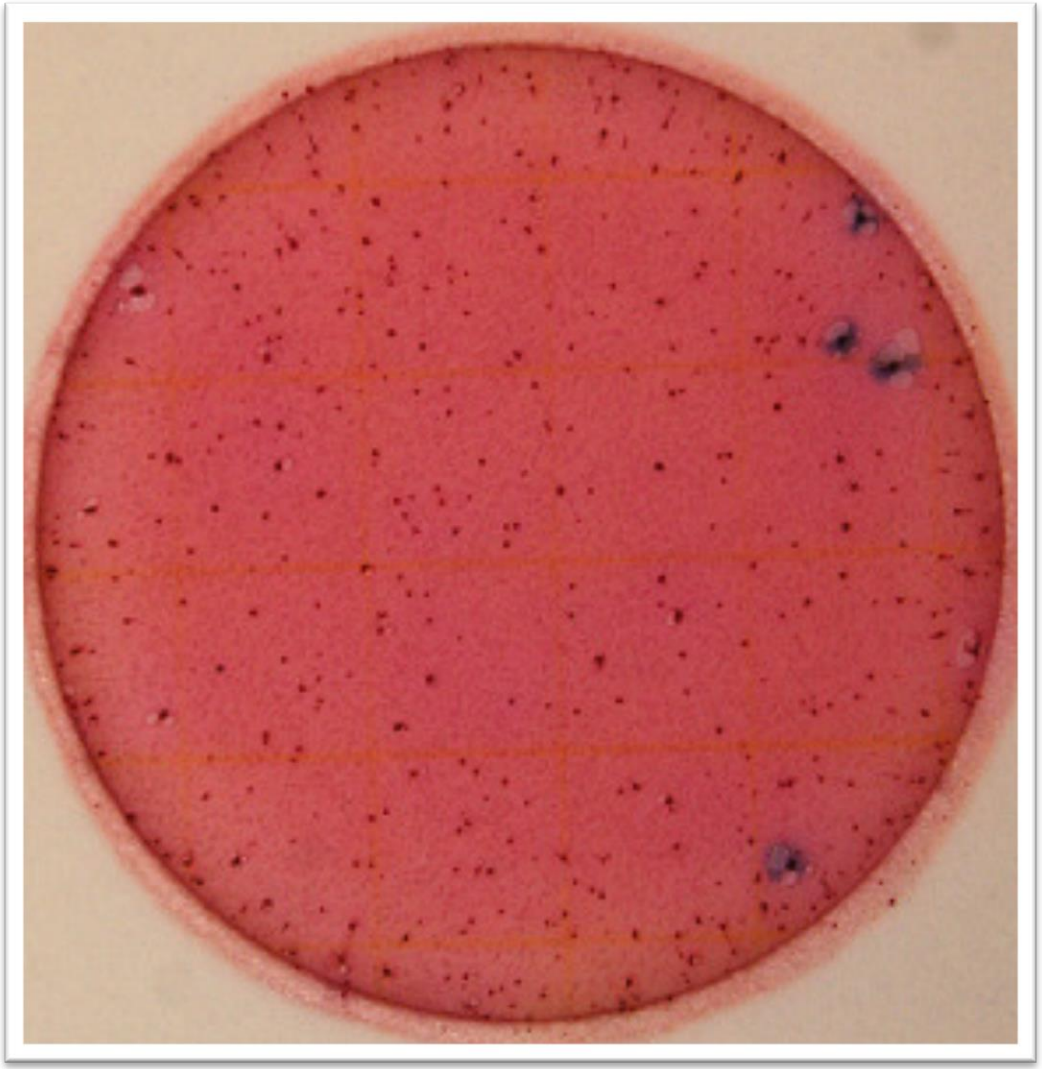


How many *E. coli* colonies can you see?

When plates turn a deep red color, or plates have many gas bubbles due to the presence of many coliforms, count ALL blue colonies as presumptive *E. coli* colonies

This is the only case where blue colonies without gas bubbles are counted

Example #12



How many *E. coli* colonies can you see?

How Do We Calculate Results from the Plates?

Plate 1

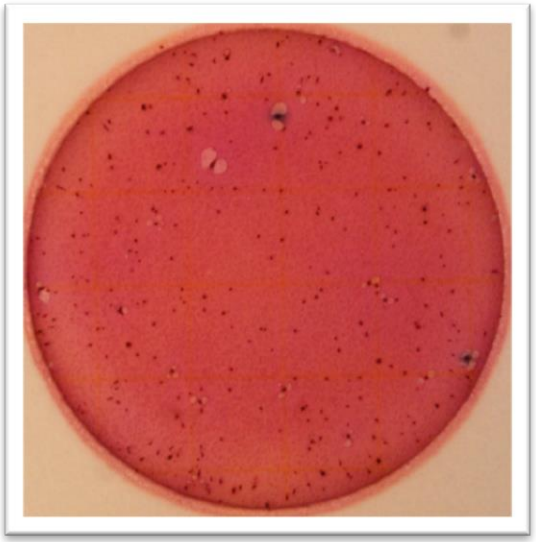


Plate 2

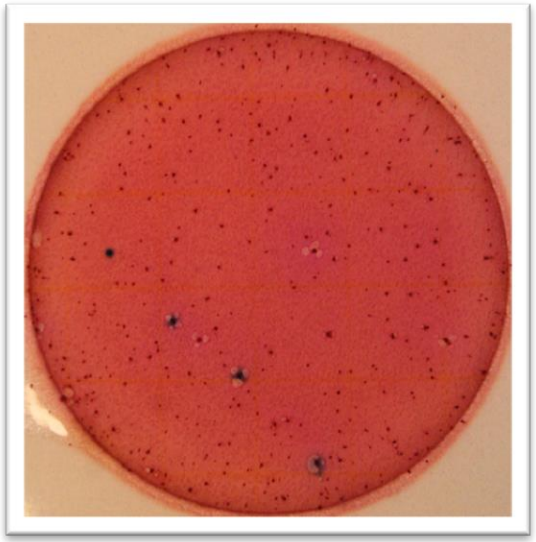


Plate 3

	Plate 1	Plate 2	Plate 3
E. coli Colonies	3	2	3

How Do We Calculate Results?

Calculate the results:

Step 1: $\dfrac{\text{Plate 1 + Plate 2 + Plate 3}}{\text{3 plates}}$ = Average CFU/1 ml

Step 2: **Average CFU/1 mL x 100 mL = # CFU/100 ml**

How Do We Calculate Results?

	Plate 1	Plate 2	Plate 3
E. coli Colonies	3	2	3

Step 1: $\frac{3 + 2 + 3}{3} = 2.67$ CFU/1 ml

Step 2: 2.67 CFU/1 ml x 100 ml = **267 CFU/100 ml**

How Do We Calculate Results from these Plates?

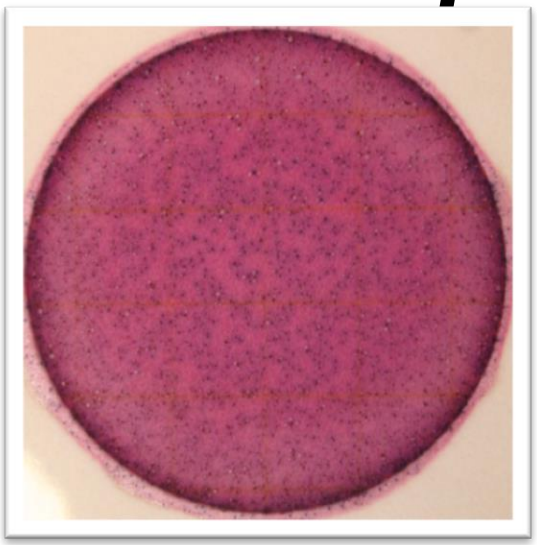


Plate 1

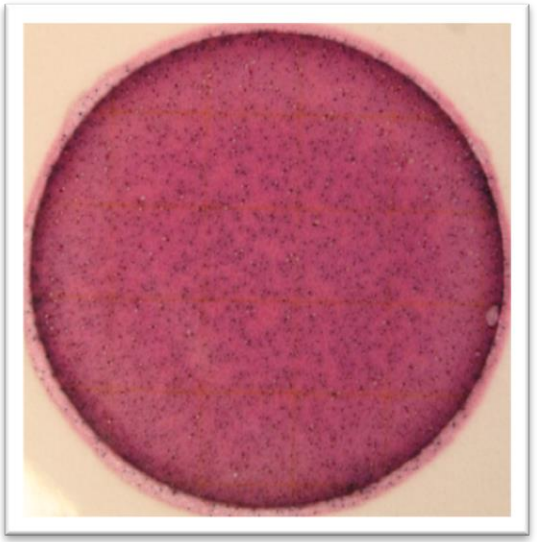


Plate 2

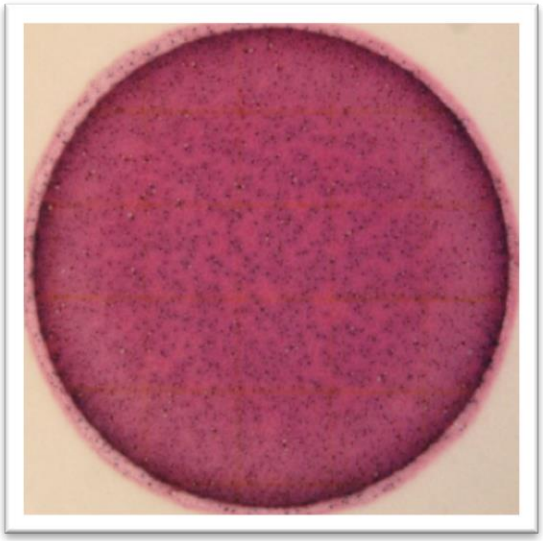


Plate 3

	Plate 1	Plate 2	Plate 3
E. coli Colonies	TNTC	TNTC	TNTC

How Do We Calculate Results?

	Plate 1	Plate 2	Plate 3
E. coli Colonies	TNTC	TNTC	TNTC

Step 1: $\frac{\text{TNTC + TNTC + TNTC}}{3} = \frac{\text{TNTC}}{\quad}$

*TNTC is generally defined as more than 150 colonies

6. Disposal and Clean-up

- **Spray plates with disinfectant (10% bleach, disinfectant spray, rubbing alcohol, etc.) and seal in bag or used Whirl-Pak before throwing away**
- Spray incubator with disinfectant to clean after use
- Remember to wear gloves – these are living bacteria colonies!
- Wash your hands when finished

Storage of Petrifilm

- If using within one month, keep in the fridge
- If not, keep them in the freezer for storage
- Remember to thaw before use!

EPA Recommended E. coli Levels

for recreational waters

	Designated swimming	*Moderate swimming area*	*Light swimming area*	*Infrequent swimming area*
E. coli (cfu/100ml)	*<235*	*<298*	*<410*	*<576*

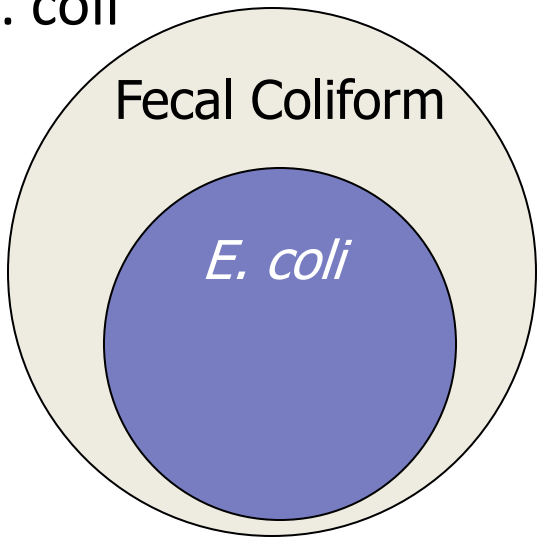
These levels correspond to an acceptable risk level of 8 people out of 1000 getting sick

(from US EPA 1986, 2002a)

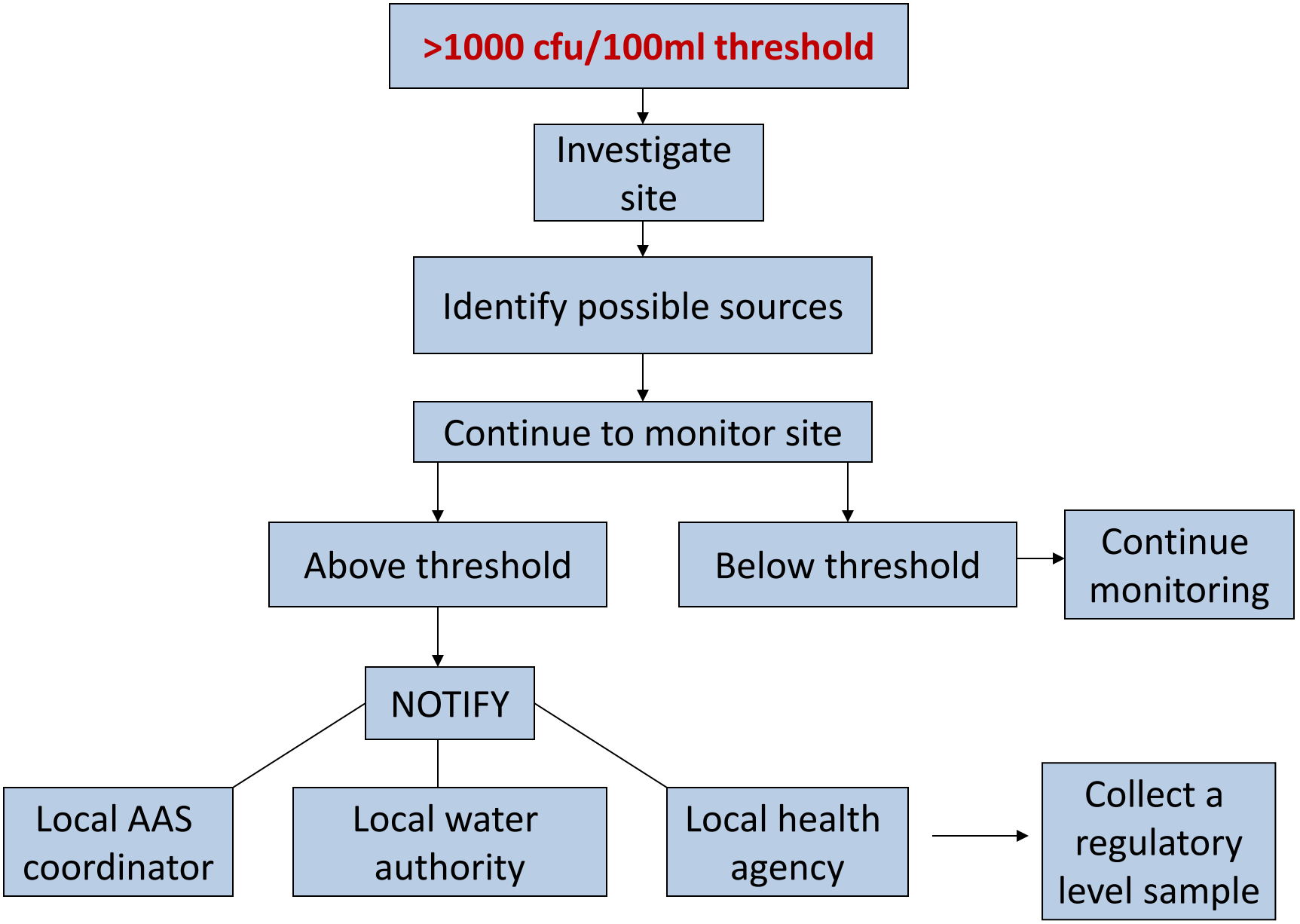
Fecal vs. E. coli

- According to the EPA, approximately 60% of fecal coliform represents *E. coli*
  - For example:
    - A fecal coliform sample that has 1000 cfu/100 ml has approximately 600 cfu/100 ml of E. coli
    - A fecal coliform sample that has 500 cfu/100 ml has approximately 300 cfu/100 ml of E. coli

But what level is AAS concerned with?



Counts that Warrant Action



GEORGIA ADOPT-A-STREAM: Bacterial Form

To be conducted every month

All monitoring programs

Bacterial specific

SITE INFORMATION

Group Name:_____ Event Date: _____ (MMDDYYYY)

Group ID: G-_____ Site ID: S-_____ Time Sample Collected:_____ (HHMM am/pm)

Stream Name:_____ Time Spent Sampling: _____ (Min)

Monitor(s):_____ Total Time Spent Traveling *(optional)* : _____ (Min)

Number of Participants:_____ Furthest Distance Traveled *(optional)* : _____(Miles)

WEATHER

Present conditions (check all that apply)

☐ Heavy Rain ☐ Steady Rain ☐ Intermittent Rain

☐ Overcast ☐ Partly Cloudy ☐ Clear/Sunny

Amount of rain, if known?

Amount in Inches :_____

In Last Hours/Days:_____

*Refer to *wunderground.com* for rainfall data

OBSERVATIONS

Flow/Water Level: *(check all that apply)* ☐ Dry ☐ Stagnant/Still ☐ Low ☐ Normal ☐ High ☐ Flow (over banks)

Water Clarity: ☐ Clear/Transparent ☐ Cloudy/Somewhat Turbid ☐ Opaque/Turbid

Water Color: ☐ No Color ☐ Brown/Muddy ☐ Green ☐ Milky/White ☐ Tannic ☐ Other:_____

Water Surface: ☐ Clear ☐ Oily Sheen: does it break when disturbed? Yes/No (circle one) ☐ Algae

☐ Foam ◯ Greater than 3" high ◯ It is white

Water Odor: ☐ Natural/None ☐ Gasoline ☐ Sewage ☐ Rotten Egg

☐ Fishy ☐ Chlorine ☐ Other:_____

Photos: Please take images to document your observations and changes in water quality conditions.
Photo point directions can be found in the manuals. Images can be submitted online with your other data.

Trash: ☐ None ☐ Yes, I did a cleanup ☐ This site needs an organized cleanup

BACTERIAL

3M Petrifilm Method: ***Escherichia coli***

Run three (3) plates/tests for each site, plus one (1) blank plate. Process within 6-24hrs, incubate at 35°C ±1° and read at 24 ± 1 hr

Plate	Colonies	Find AVG of Number of Colonies (total # colonies/total # of plates (do not include blank))	cfu/100mL
Blank			
1		(/) x 100 =	
2			
3			
Total # Colonies			

Sample Holding Time (HH): _____

Date START(MMDDYYYY): _____ Date END (MMDDYYYY):_____

Time START (HHMM):_____ Time END (HHMM):_____

MIN Temp (°C):_____ MAX Temp (°C): _____

COMMENTS

Any changes since you last sampled at this site? If yes, please describe.

Please submit data to our online database at www.AdoptAStream.Georgia.Gov

Observations

- Flow/Water Level
- Water Clarity
- Water Color
- Water Surface
- Water Odor
- Photos
- Trash

OBSERVATIONS

Flow/Water Level: *(check all that apply)*	☐ Dry ☐ Stagnant/Still ☐ Low ☐ Normal ☐ High ☐ Flood (over banks)
Water Clarity:	☐ Clear/Transparent ☐ Cloudy/Somewhat Turbid ☐ Opaque/Turbid
Water Color:	☐ No Color ☐ Brown/Muddy ☐ Green ☐ Milky/White ☐ Tannic ☐ Other:_____
Water Surface:	☐ Clear ☐ Oily sheen: Does it break when disturbed? Yes/No (circle one) ☐ Algae ☐ Foam ◯ Greater than 3" high ◯ It is pure white Other:_____
Water Odor:	☐ Natural/None ☐ Gasoline ☐ Sewage ☐ Rotten Egg ☐ Fishy ☐ Chlorine ☐ Other:_____
Photos:	Please take images to document your observations and changes in water quality conditions. Photo point directions can be found in the manuals. Images can be submitted online with your other data.
Trash:	☐ None ☐ Yes, I did a cleanup ☐ This site needs an organized cleanup

Bacterial Data Form

- Use Bacterial data form (Chemical/Bacterial combo data form may also be used)
- Fill in number of colonies for each plate
  - Blank count should be zero
- Volunteers can send scanned color images of plates to AAS or their local coordinator if they need assistance with counting colonies

BACTERIAL

3M Petrifilm Method: *Escherichia coli*

Run three (3) plates/tests for each site, plus one (1) blank plate. Process within 6-24hrs, incubate at 35°C ±1° and read at 24 ± 1 hr

Plate	Colonies		Find AVG of Number of Colonies (total # colonies/total # of plates (do not include blank)	cfu/100mL
Blank				
1			(/) x 100 =	
2		Sample Holding Time (HH): _____		
3		Date START(MMDDYYYY): _____	Date END (MMDDYYYY):_____	
Total # Colonies		Time START (HHMM):_____	Time END (HHMM):_____	
		MIN Temp (^{0}C):_____	MAX Temp (^{0}C): _____	

Submit the Data

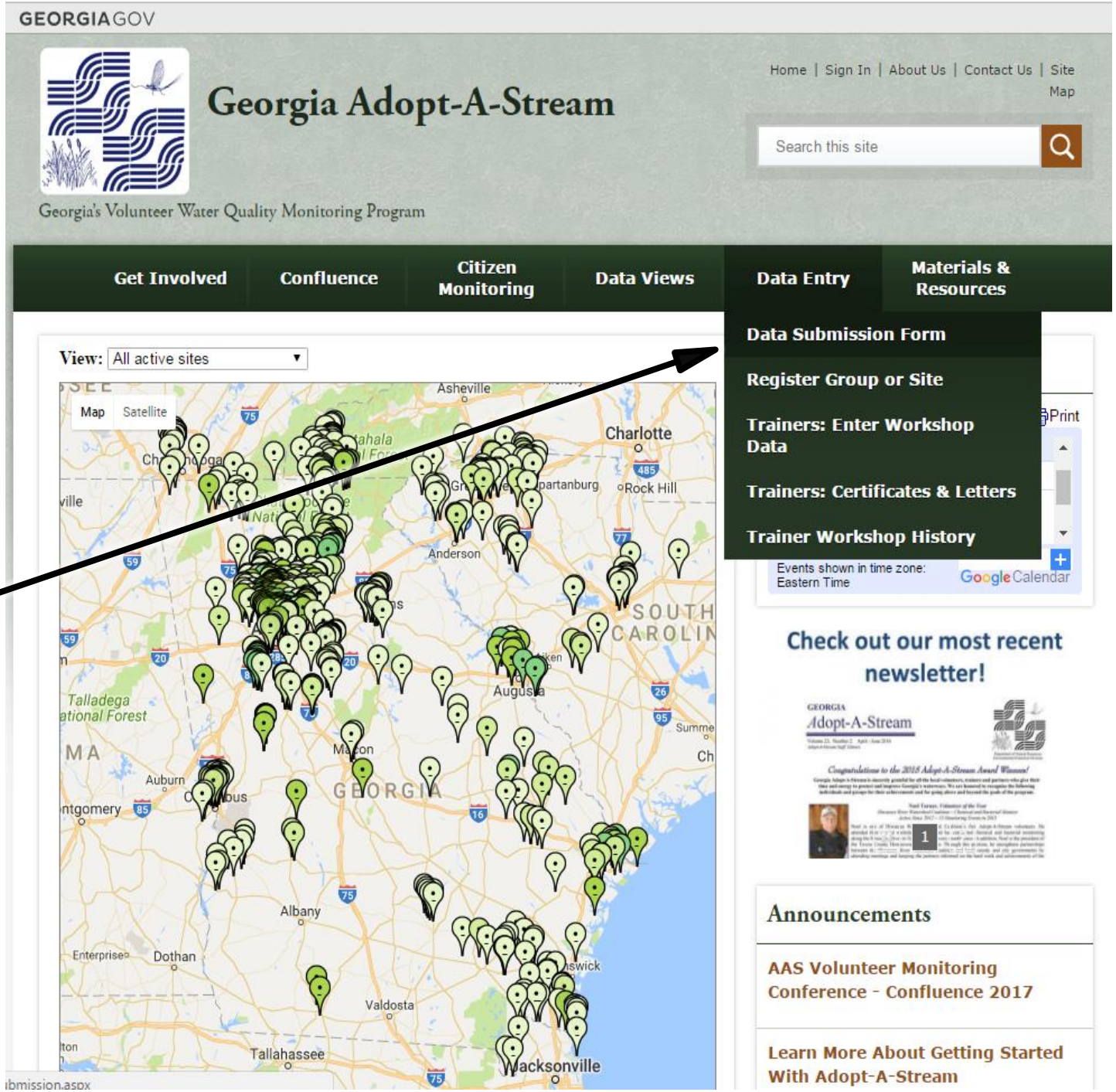
As soon as possible after monitoring is complete

Data should be submitted to the state program's **online database**:

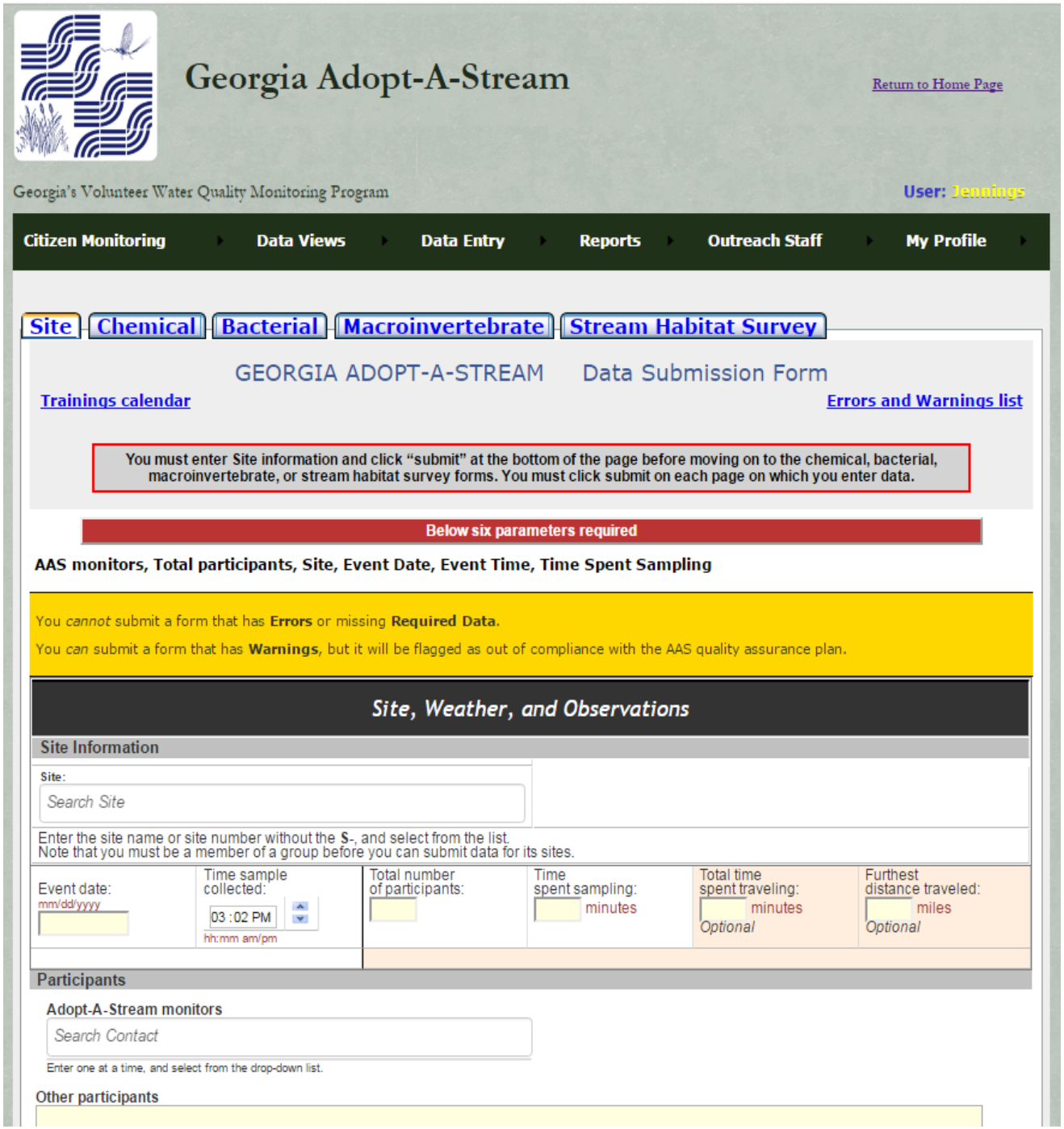
AdoptAStream.Georgia.gov

Share your data with partners, local governments and your local Adopt-A-Stream coordinators

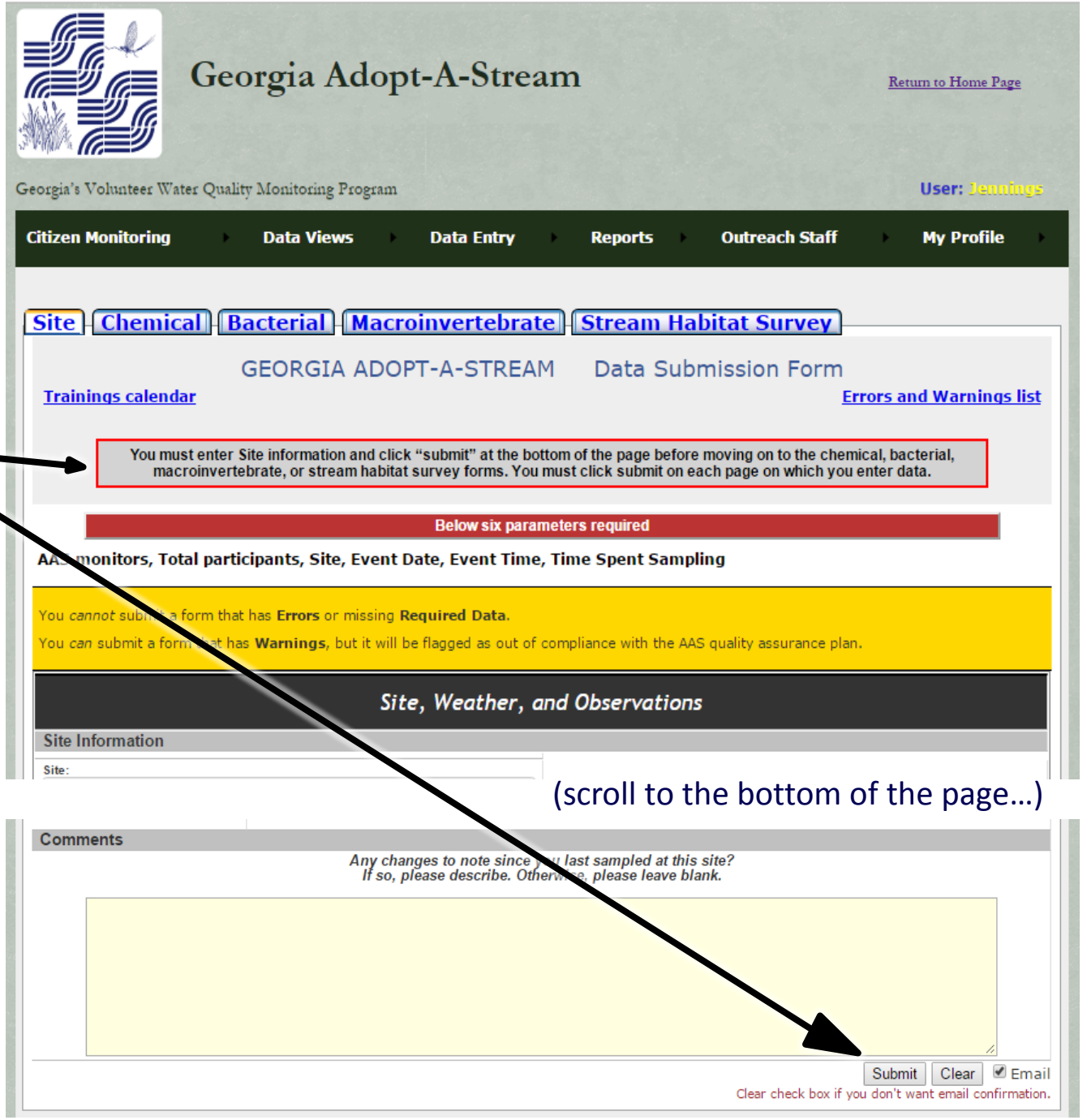
From the website's Home Page, select "Data Submission Form" under the Data Entry tab.



Enter your site information as well as any weather and observation information on this page.

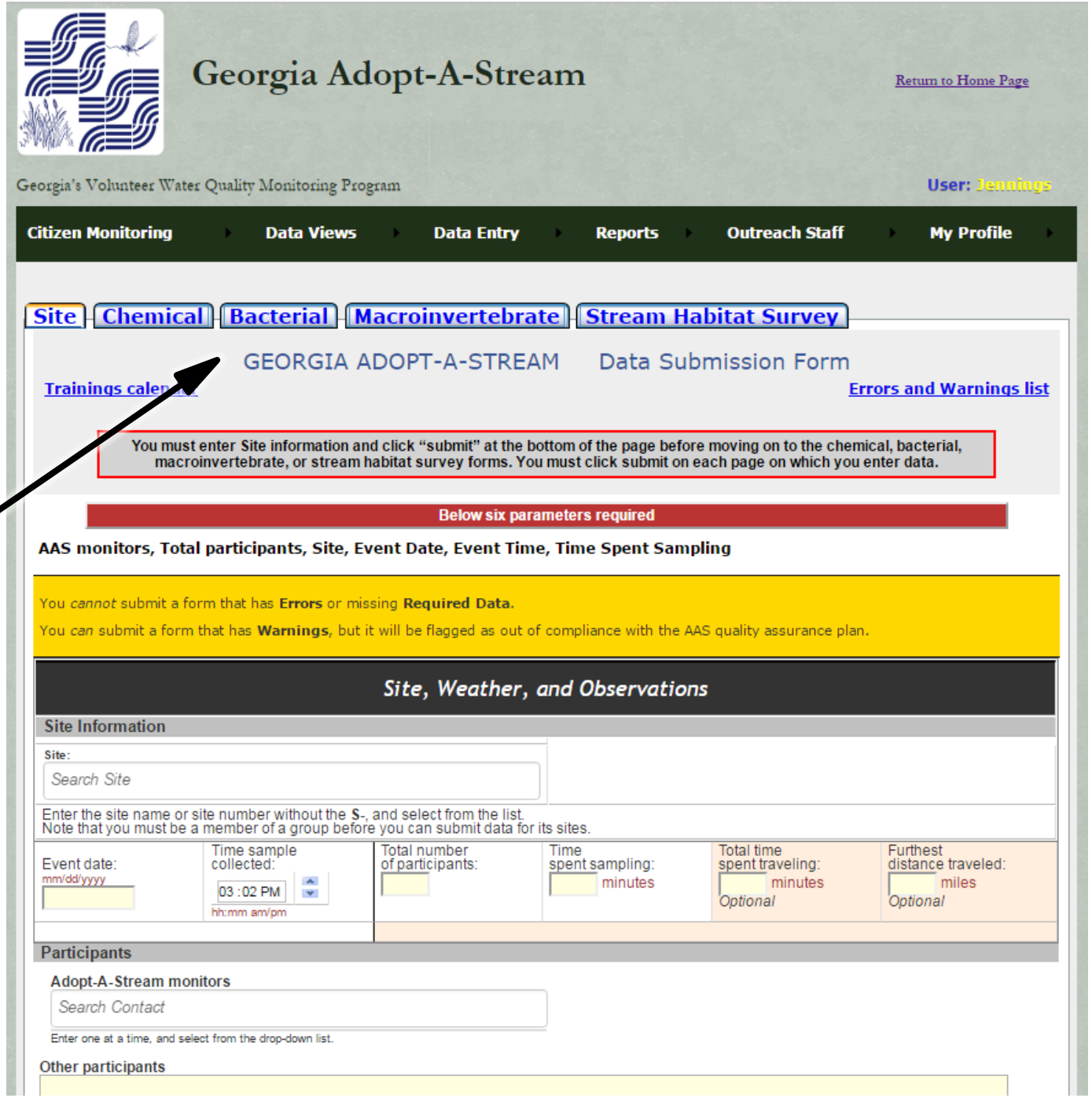


Click “Submit” at the bottom of the page to record your data. You must submit your site data before you can enter bacterial data

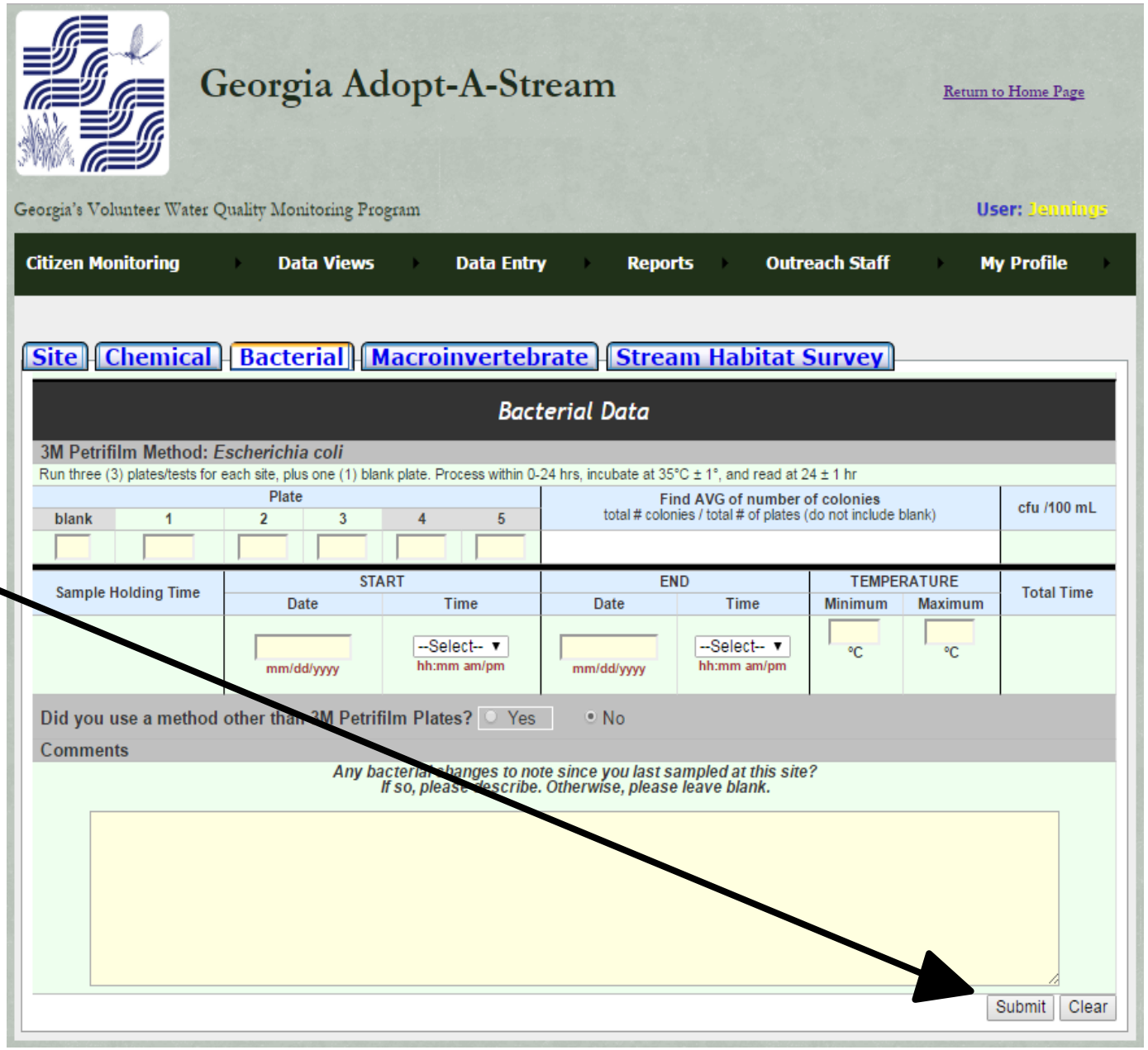


(scroll to the bottom of the page…)

After clicking "Submit," click on the Bacterial tab to continue entering data

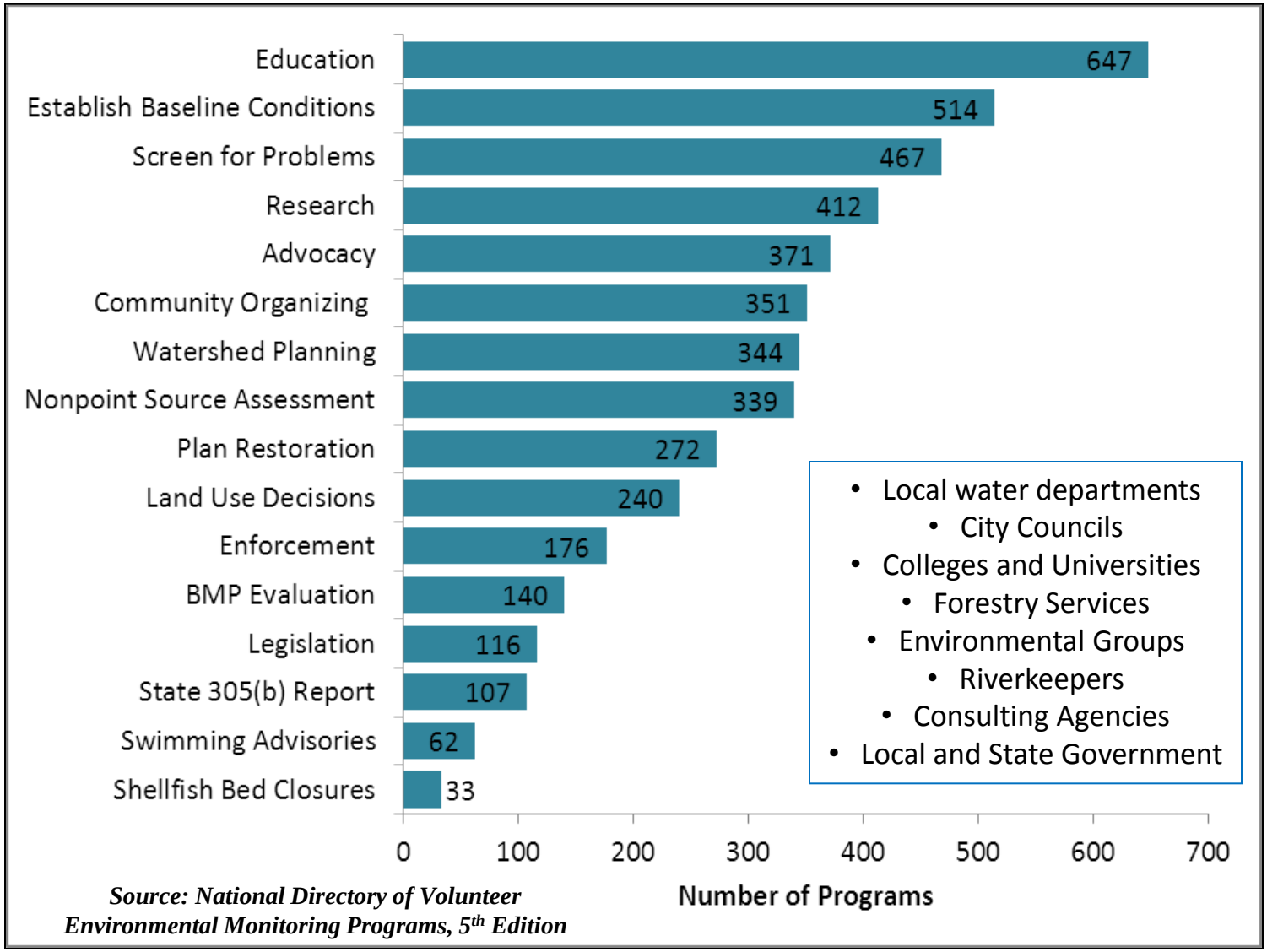


Fill out the form and click “Submit” to record your bacterial data!



Note: You must click “Submit” on each tab if you are entering data for multiple parameters

Volunteer Monitoring Data Uses



Source: National Directory of Volunteer Environmental Monitoring Programs, 5th Edition

Just the Facts

- *Raise **A**wareness*
- *Water quality **D**ata*
- *Gather **O**bservations*
- *Encourage **P**artnerships*
- *Provide **T**ools and **T**raining*

Quickly assess health risks due to bacterial contamination of surface waters

Single-celled, living microorganisms

1000 cfu/100 ml

- *Wildlife*
- *Livestock*
- *Urban storm runoff*
- *Leaking pipes*
- *Failing septic systems*

CFU/100 mL – colony forming units/100 mL

1 blank + triplicate of sample = 4 plates

Purpose of a blank?

Once a month

Place samples in cooler with ice

Rainstorm

UV light will reduce bacteria levels

Spray plate with disinfectant and seal in plastic bag for disposal

Warmer temps = higher E. coli survival rates

Cooler temps = lower E. coli survival rates

***General coliforms** are red in color with entrapped gas bubbles, while **E. coli** are blue in color with entrapped gas bubbles*

asap, but no more than 24 hours after collection

35°C ± 1 degree for 24 hours ± 1 hour