

# Equipment

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



# Lessons Learned

## Planning

- Remember that supplies take up a lot of space initially; Plan for necessary storage space (in fridge and freezer too)!
- 3M Petrifilm spreaders can take a few weeks to arrive if buying in bulk



# Lessons Learned

## Orientation and Project Commitment

- Get a firm commitment from interested volunteers and set training dates early in season
- Require attendance at entire training



# Lessons Learned

## Equipment and Inventory

- Provide all supplies volunteers will need in an organized “kit”
- Don’t give out too many supplies initially; Wait to see commitment level of each volunteer
- Plan for an initial inventory and a mid-summer re-supply



# Lessons Learned

## Communication and Feedback

- Stress the need for timely data submission
- Stress the importance of filling out data sheets completely
- Check data sheets as they arrive
- Visit volunteers at least once during the season
- Report back to volunteers 2-3 times during the summer

**CITIZENS MONITORING BACTERIA DATA SHEET**

Date \_\_\_\_\_ Volunteer ID \_\_\_\_\_ Current Weather \_\_\_\_\_  
Collection Time \_\_\_\_\_ (am/pm) Site ID \_\_\_\_\_  Partly  Overcast  Snow  Rain  Storm  
Certified Member's Name \_\_\_\_\_ Worst Weather in Past 48 hrs. \_\_\_\_\_  
Stream River Name \_\_\_\_\_  Partly  Overcast  Snow  Rain  Storm


Stream Flow \_\_\_\_\_ Air Temp \_\_\_\_\_ (°C) Transparency \_\_\_\_\_ (cm) or \_\_\_\_\_ (ft) (°F)  
 High Water Temp \_\_\_\_\_ (°C)  Normal  Low  
Stream assessment comments and observations: \_\_\_\_\_

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

Test Method	Sample Volume (mL)	# <i>E. coli</i> counted @ 24 hours	# <i>E. coli</i> counted @ 24 hours CFU/100mL	# <i>E. coli</i> counted @ 48 hours	# <i>E. coli</i> counted @ 48 hours CFU/100mL	Incubation Temperature _____ °C
EASYGEL - Sample 1	A			A		Time Samples Placed in Incubator _____
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 (or red/blue) colonies with gas

Bacteria analysis comments & observations (include time samples counted if different from 24 or 48 hours): \_\_\_\_\_

 Citizens Monitoring Bacteria  
Revised 10/10/08



# Lessons Learned

## Making it Easy for the Volunteers

- Provide postage paid envelopes for returning data sheets
- Provide extra copies of data sheets
- Laminate field and lab methods
- Minimize number of ID #s, codes, you ask volunteers to use



# Site Selection

- Choose sites based on project goals
- Consider
  - Tributaries or other inflows to the stream
  - Land uses alongside the stream
- Also consider:
  - Budget
  - Number of volunteers
  - Safety
  - Access
    - Get written permission if monitoring on private land.  
Know state laws.



# Site Selection Resources

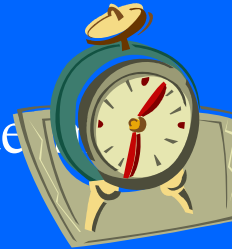
- EPA's Volunteer Stream Monitoring Methods Manual <http://www.epa.gov/volunteer/stream/stream.pdf>
- Washington Dept. of Ecology's, "A Citizen's Guide to Understanding and Monitoring Streams and Lakes"  
<http://www.ecy.wa.gov/Programs/wq/plants/management/joysmanual/selectingstreamlocations.html>





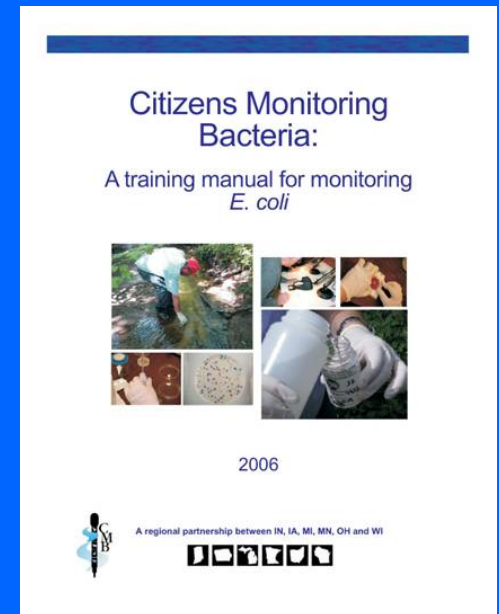
# Frequency of Monitoring

- Choose frequency based on project goals
  - Check your state or 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

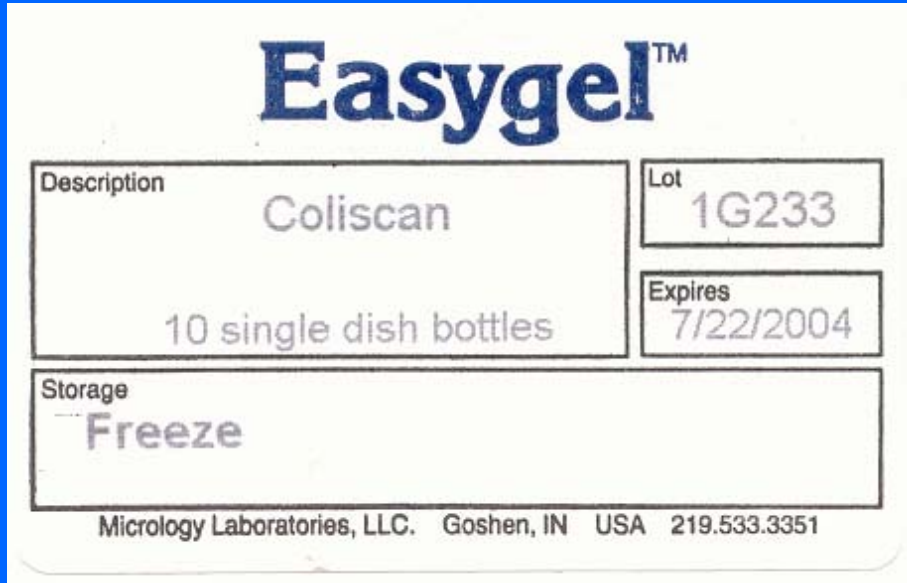


# Quality Assurance

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



# Quality Assurance



Expiration dates  
on bottle and box  
that media comes  
in



Expiration date on bag  
Petri dishes come in →  
store at room temp  
and keep bag sealed  
once opened



# Quality Control

- Ensure samples are collected consistently and accurately
  - Field blanks
  - Field and lab replicates
  - Control plates
  - Split samples
  - Regular inspection of equipment



# Resource about QA/QC

- **Building Credibility: Quality Assurance and Quality Control for Volunteer Monitoring Programs**

<http://www.usawaterquality.org/volunteer/Outreach/BuildingCredibilityVI.pdf>

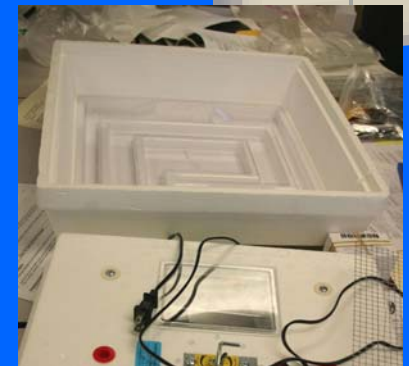
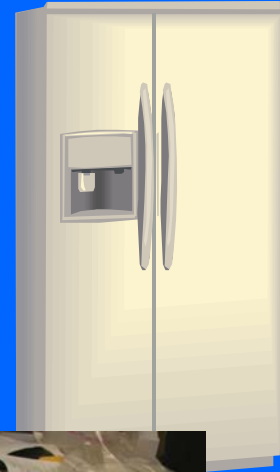




**Field sampling and  
preparing the test  
plates**

# Field Sampling – Before you go

- Take Petrifilm out of fridge to warm to room temperature
- Take Easygel out of freezer to thaw
- Fill trays in incubator with distilled water
- Turn on incubator to 35°C
- Collect supplies, including 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: \_\_\_\_/\_\_\_\_/\_\_\_\_ Volunteer ID: \_\_\_\_\_  
 Collection Time: \_\_\_\_:\_\_\_\_ (am/pm) Site ID: \_\_\_\_\_  
 Certified Monitor's Name: \_\_\_\_\_  
 Stream/River Name: \_\_\_\_\_

Current Weather  
 Clear/Sunny  Overcast  Showers  Rain (Steady)  Storm  
 Worst Weather in Past 48 hrs.  
 Clear/Sunny  Overcast  Showers  Rain (Steady)  Storm

Stream Flow:  High  Normal  Low  
 Air Temp: \_\_\_\_\_ (°C)  
 Water Temp: \_\_\_\_\_ (°C)  
 Transparency: \_\_\_\_\_ (cm) or \_\_\_\_\_ (NTU) (optional)

Stream assessment comments and observations:

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

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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 (or red/blue) colonies with gas

Incubation Temperature: \_\_\_\_\_ °C  
 Time Samples Placed in Incubator: \_\_\_\_\_

Bacteria analysis comments & observations (include time samples counted if different from 24 or 48 hours):

Citizens Monitoring Bacteria  
 Revised 3/27/2006

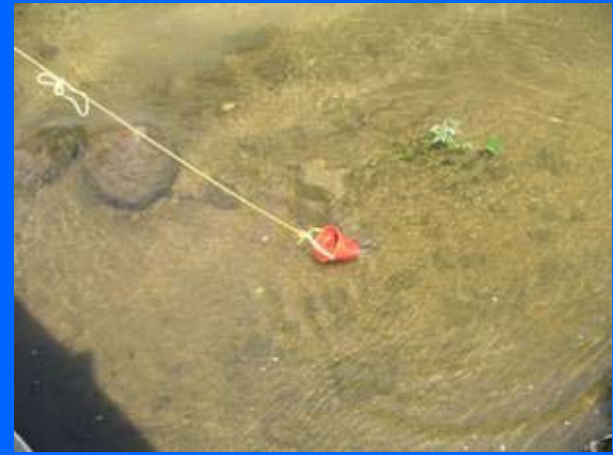
# Collecting a Sample in the Stream

- Rinse collection bottle 3 times
  - Cover and shake well when rinsing
- Dip the bottle into the water upside down and pointing upstream
  - Keep your hands away from lip & lid
- Tip it up and allow to fill completely; cap it
- If splitting sample, shake bottle well before pouring



# Sampling from a bridge

- Use a sterile bucket if possible
- If not possible, the following steps will help prevent cross-contamination:
  - Drop bucket into the stream and allow to fill, pull it up
  - Shake it well to rinse; rinse 3 times
  - Fill again, and use it to rinse the collection bottle 3 times
  - THEN fill the bucket again, and from that fill the collection bottle



# 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



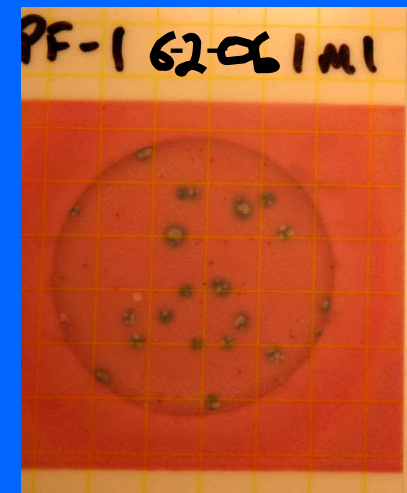
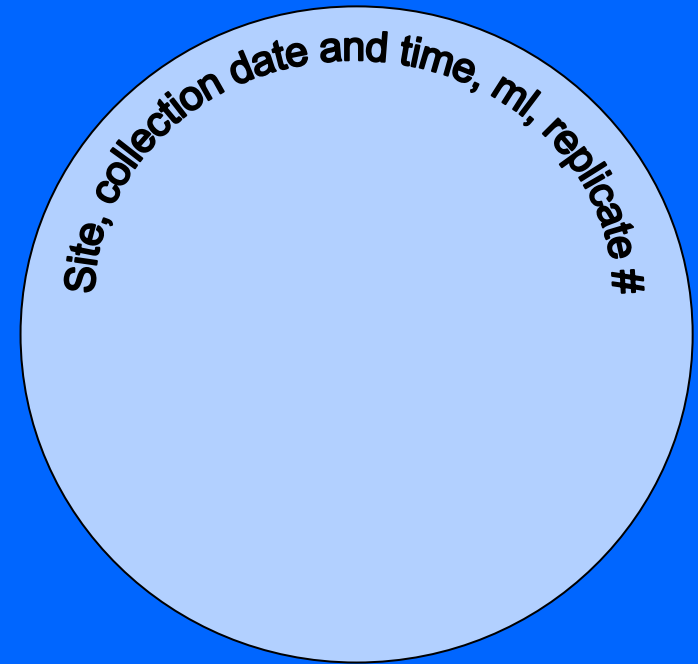
# Preparing to plate samples

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



# Preparation continued

- Label the bottom Petri dishes along the edge, and at the front top of Petrifilm using a permanent marker
  - Label should include:
    - site name
    - date and time of collection
    - volume of water plated (ml)
    - replicate #, if any
- Why label the bottom of dish?



# Coliscan Easygel Plating

- Carefully remove pipette from sterile paper (keep sterile)
- Transfer 1 ml to 5 ml of water from collection container (after mixing) to Easygel media bottle
  - Label your media bottle with # of mls used & mix bottle
- Pour media into labeled petri dish

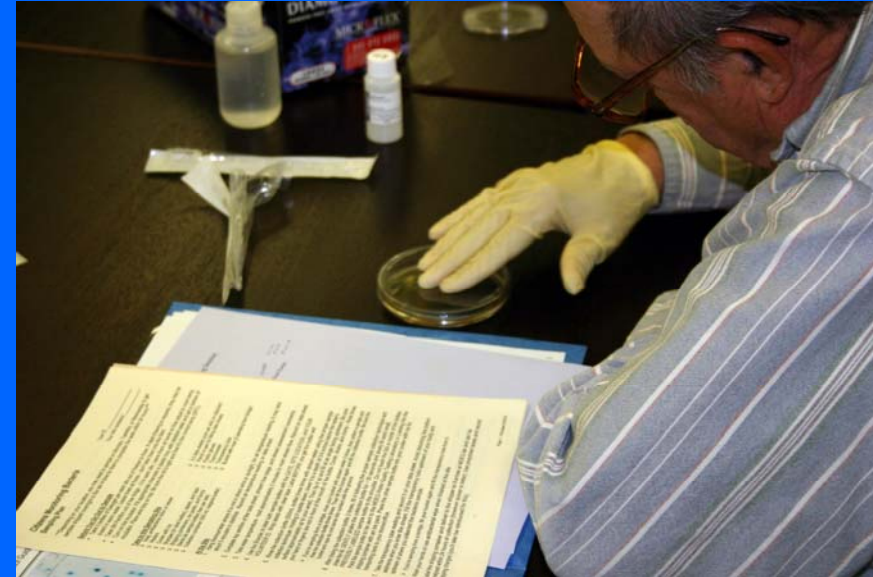




- Swirl the dish (figure-8 mode) gently to evenly distribute the media (be careful not to splash over the side or get any on the lid)
- Let plates sit undisturbed at room temperature (out of direct sunlight) for 45 minutes or until solid

**STOP HERE UNTIL MEDIA IS SOLID**

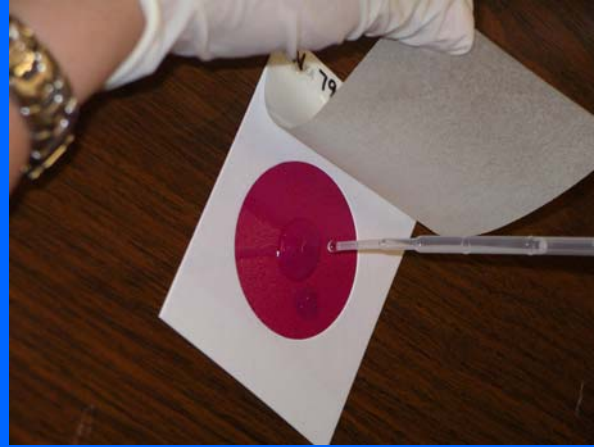
- After media gels, add to incubator, upside down (to reduce condensation on plate bottom). They can be stacked on top of one another



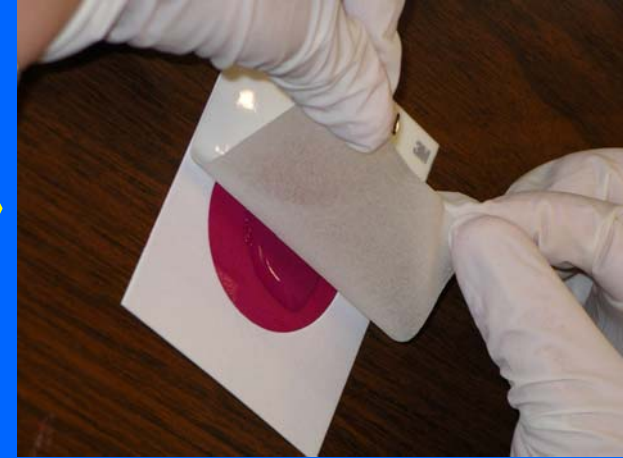
# Plating 3M™ Petrifilm™



1. Pipette water sample (1 ml only)



2. Lift film and dispense sample onto pink



3. Carefully roll down film

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

5. Let sit 1 minute

4. Gently use spreader, if needed



- 24 hours later....



# Reading & Interpreting Easygel Plates

- Count after 24 hours of incubation
- Optional: Count again after 48 hours
- Count dark blue or deep purple colonies
- Teal and pink colonies are not *E. coli*



# Coliscan Easygel

Contains 2 enzymes specific chemicals (chromogens)

- “Red Gal” (galactosidase)
  - Red or pink color produced
- “X-Gluc” (glucuronidase)
  - Teal color produced
- Only *E. coli* produces both enzymes



**Red + Teal = Dark Blue/Purple**

Is this an E. coli colony?

**NO**

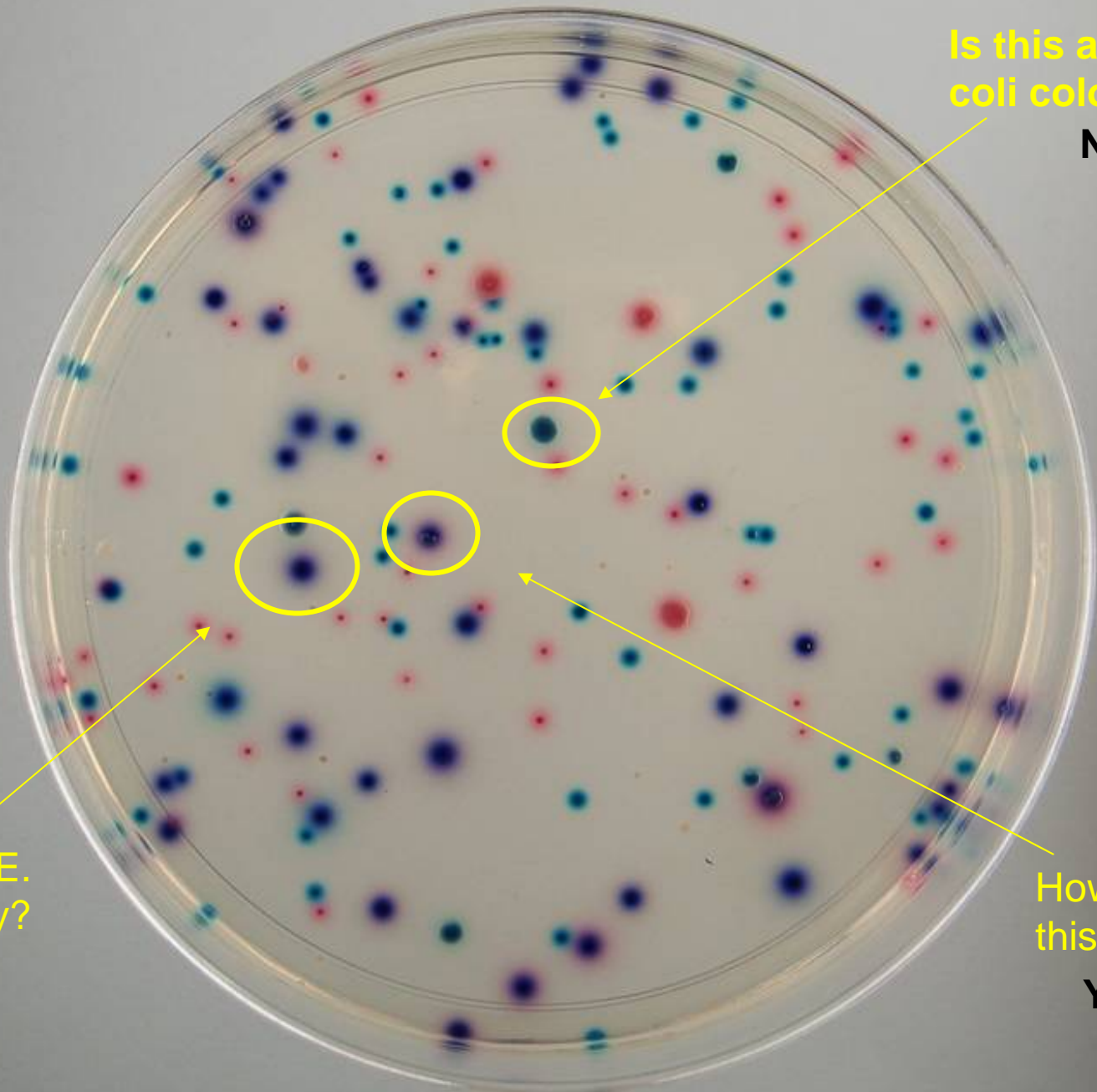


Is this an E. coli colony?

**YES**

How about this one?

**YES**



# Reading & Interpreting Petrifilm Plates

- Count after 24 hours incubation
- Count blue colonies WITH gas bubbles
- 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

05-03 KB



# Disposing of plates

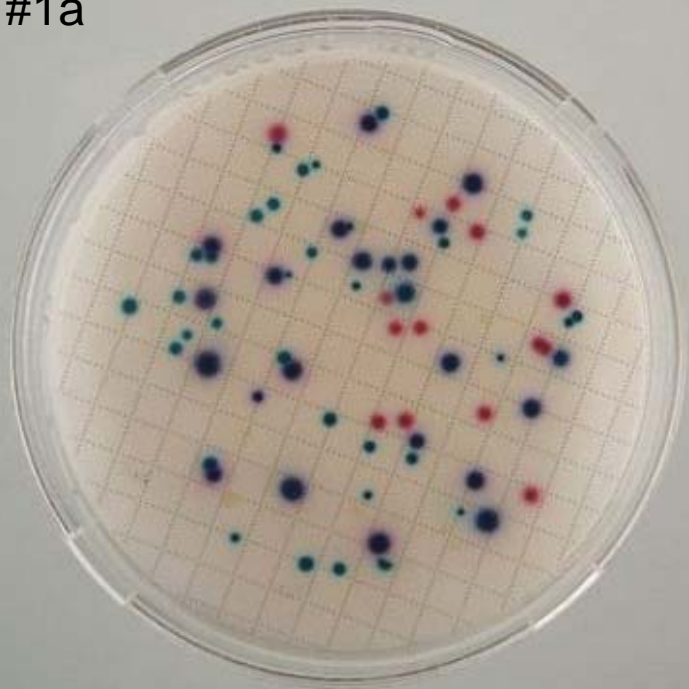
- Add a teaspoon of bleach to each Easygel Petri dish
- Cover and place in a ziplock bag
- Put Petrifilms in the same ziplock bag
- Zip tightly and throw in trash
- Dump remaining water sample and throw sample bottle in trash



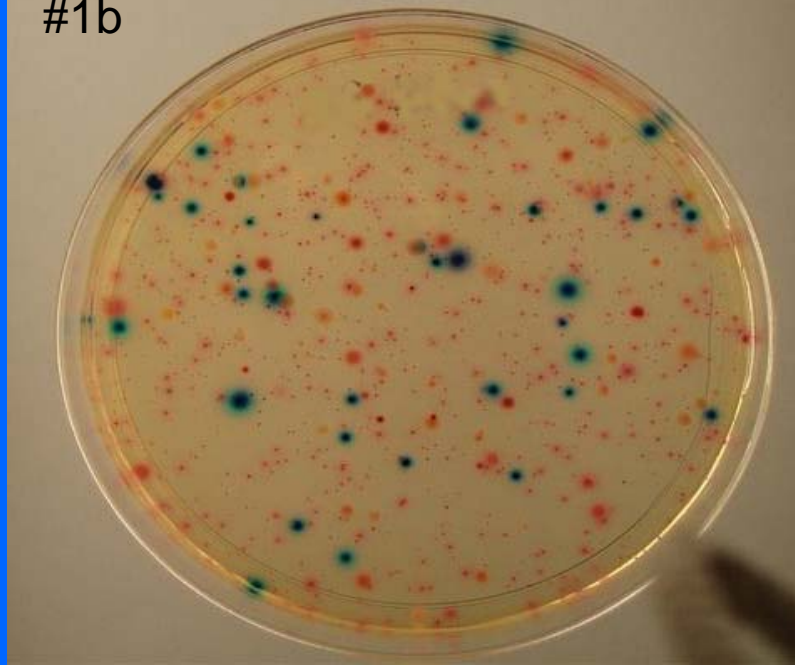
# Observations and colony counting



#1a

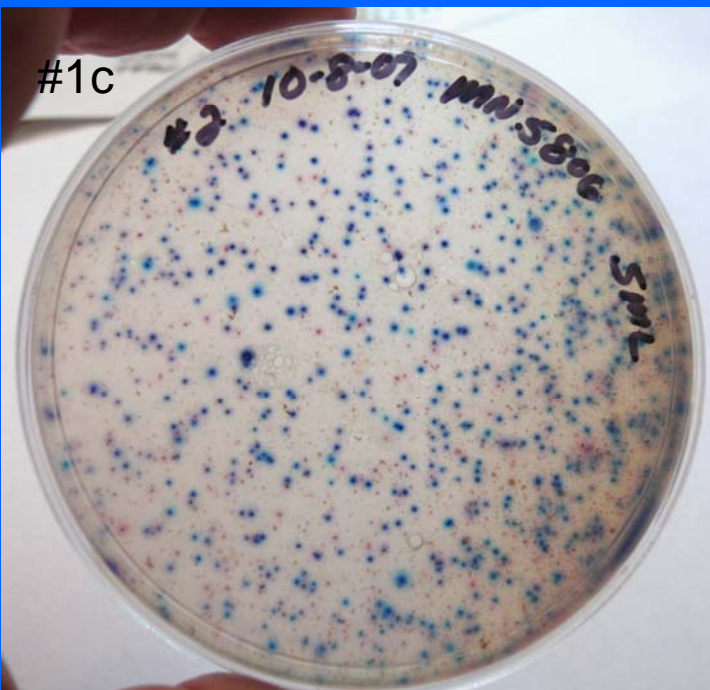


#1b

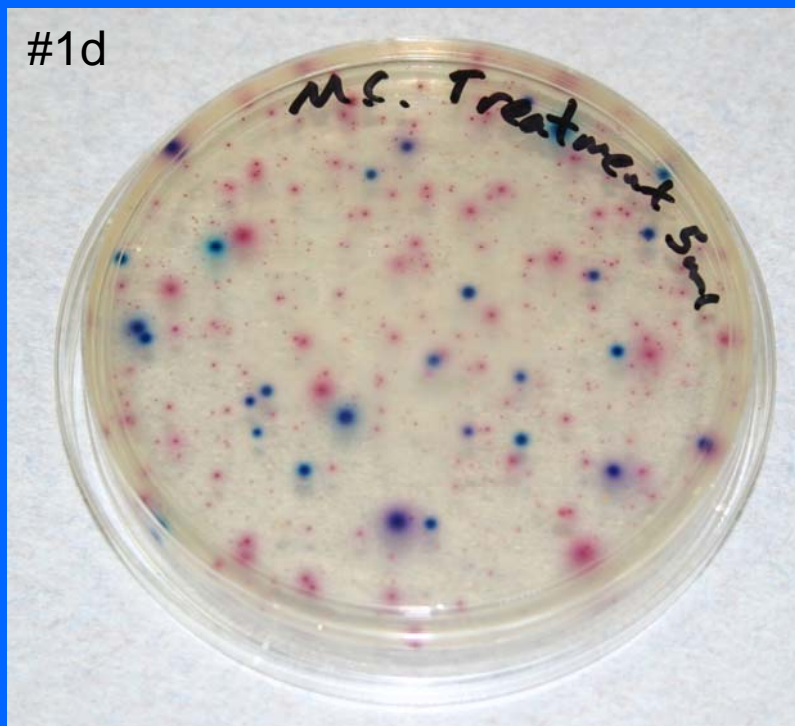


Coliscan  
Easygel7  
Plate  
Samples

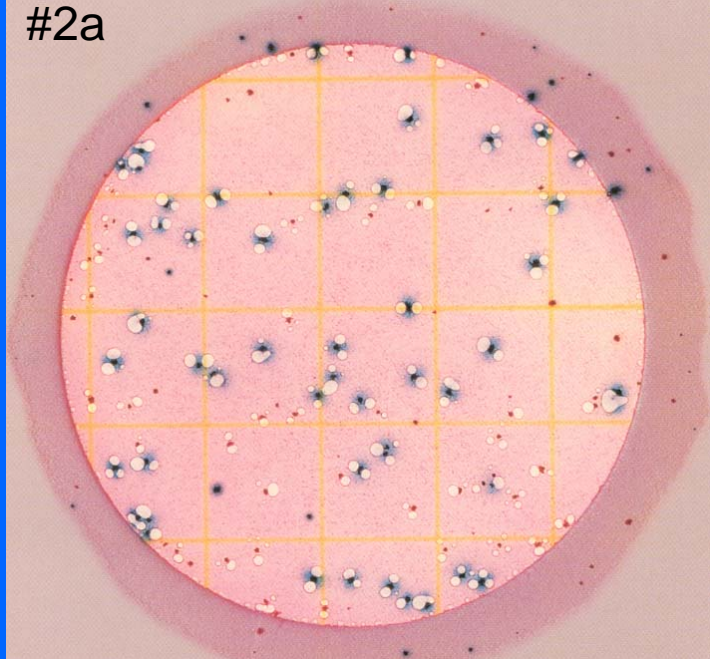
#1c



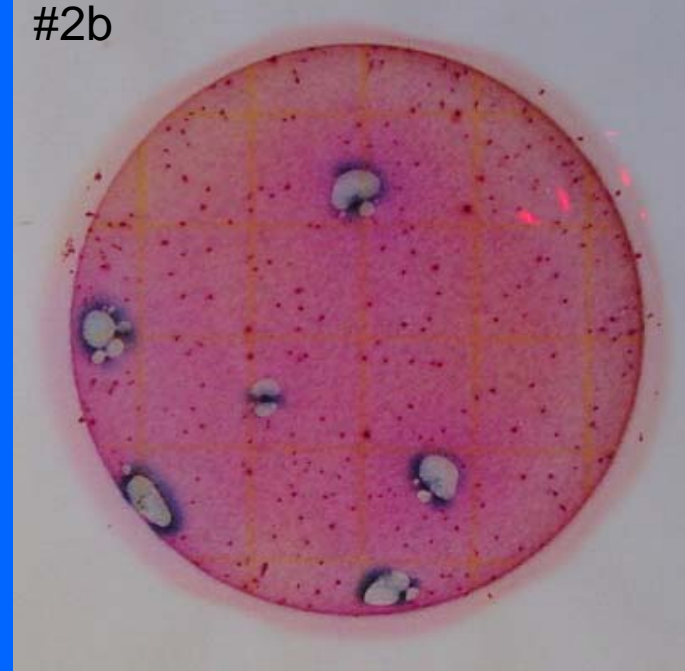
#1d



#2a



#2b

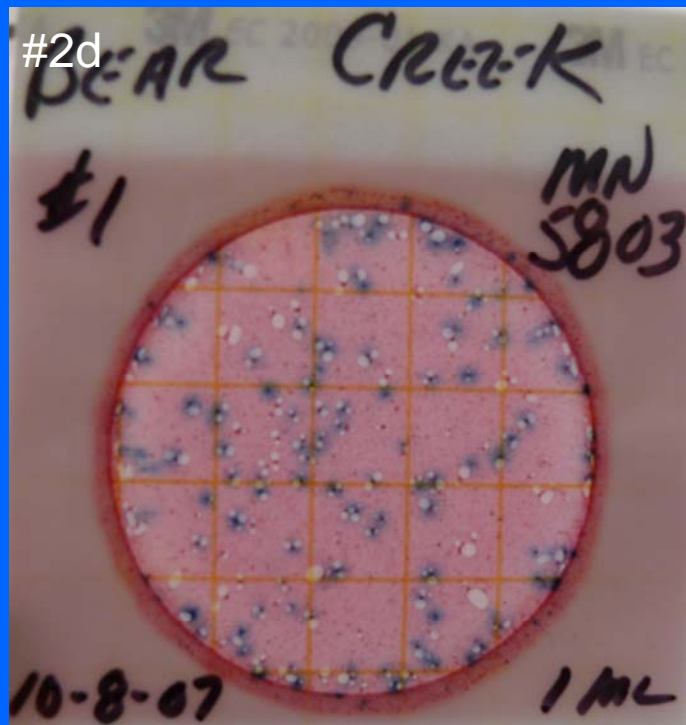


3M Petrifilm™  
Sample Plates

#2c



#2d



# CITIZENS MONITORING BACTERIA DATA SHEET

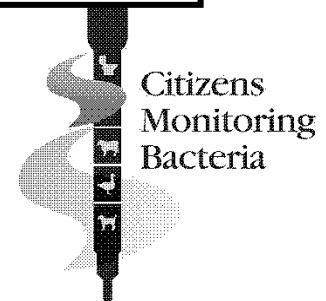
Date ____/____/____	Volunteer ID _____	Current Weather
Collection Time ____:____ (am/pm)	Site ID _____	<input type="checkbox"/> Clear/Sunny <input type="checkbox"/> Overcast <input type="checkbox"/> Showers <input type="checkbox"/> Rain (Steady) <input type="checkbox"/> Storm
Certified Monitor's Name _____		Worst Weather in Past 48 hrs.
Stream/River Name _____		<input type="checkbox"/> Clear/Sunny <input type="checkbox"/> Overcast <input type="checkbox"/> Showers <input type="checkbox"/> Rain (Steady) <input type="checkbox"/> Storm

Stream Flow	Air Temp _____ (°C)	Transparency _____ (cm) or _____ (NTU)	
<input type="checkbox"/> High			
<input type="checkbox"/> Normal			
<input type="checkbox"/> Low			
Stream assoc			
For each met placed in the			
T			
EASY			
EASY			
EASY			
3M Pet			
3M Pet			
3M Petrifilm – Sample 3	B		B

- Convince volunteers to fill out data sheet completely
- Having both site ID and description provides verification
- Collection time confirms sample reached lab within 24 hrs
- Weather, flow, and transparency provide context
- Additional comments boost understanding of conditions
- Time put in incubator makes sure of proper timing
- Calculations ...

**A** = dark blue-purple colonies; **B** = blue (or red/blue) colonies with gas

Bacteria analysis comments & observations (include time samples counted if different from 24 or 48 hours):



# Counting Easygel plates

- Use the laminated pages
- Count 1d
- 2 mls of sample
- Fill in the data sheet



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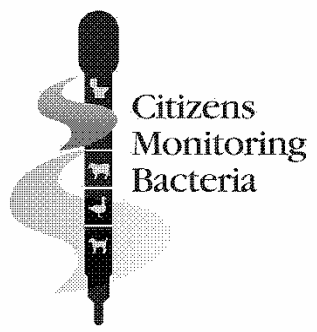
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Incubation Temperature \_\_\_\_\_ °C

Time Samples Placed in Incubator

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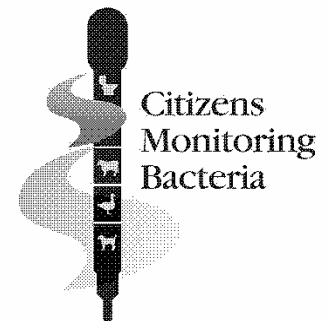
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EASYGEL – Sample 2		A		A	
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3M Petrifilm – Sample 3					

Incubation Temperature \_\_\_\_\_ °C

Time Samples Placed in Incubator

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$$100 \text{ mls} / 2 \text{ mls sample} = 50$$

$$50 \times 5 = 250 \text{ cfu}/100 \text{ mls sample}$$

Bacteria and



# Counting Petrifilm plates

- Use the laminated pages
- Count 2c
- 1 ml of sample
- Fill in the data sheet



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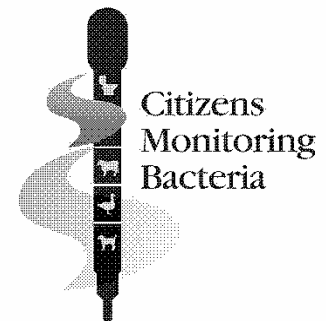
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Stream assessment comments and observations:

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

Test Method	Sample Volume (mL)	# <i>E. coli</i> counted @ 24 hours	# <i>E. coli</i> CFU /100mL @ 24 hours	# <i>E. coli</i> counted @ 48 hours	# <i>E. coli</i> 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 (or red/blue) colonies with gas

Incubation Temperature \_\_\_\_\_ °C

Time Samples Placed in Incubator

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Bacteria analysis comments & observations (include time samples counted if different from 24 or 48 hours):



# CITIZENS MONITORING BACTERIA DATA SHEET

Date ____/____/____	Volunteer ID _____	Current Weather
Collection Time ____:____ (am/pm)	Site ID _____	<input type="checkbox"/> Clear/Sunny <input type="checkbox"/> Overcast <input type="checkbox"/> Showers <input type="checkbox"/> Rain (Steady) <input type="checkbox"/> Storm
Certified Monitor's Name _____		Worst Weather in Past 48 hrs.
Stream/River Name _____		<input type="checkbox"/> Clear/Sunny <input type="checkbox"/> Overcast <input type="checkbox"/> Showers <input type="checkbox"/> Rain (Steady) <input type="checkbox"/> Storm

Stream Flow	Air Temp _____ (°C)	Transparency _____ (cm) or _____ (NTU)
<input type="checkbox"/> High		<i>(optional)</i>
<input type="checkbox"/> Normal	Water Temp _____ (°C)	
<input type="checkbox"/> Low		

Stream assessment comments and observations:

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

Test Method	Sample Volume (mL)	# <i>E. coli</i> counted @ 24 hours	# <i>E. coli</i> CFU /100mL @ 24 hours	# <i>E. coli</i> counted @ 48 hours	# <i>E. coli</i> CFU /100mL @ 48 hours
EASYGEL – Sample 1		A		A	
EASYGEL – Sample 2		A		A	
EASYGEL – Sample 3		A		A	
3M Petrifilm – Sample 1	1	B	17	B	
3M Petrifilm – Sample 2		B	1700	B	
3M Petrifilm – Sample 3					

Incubation Temperature \_\_\_\_\_ °C

Time Samples Placed in Incubator

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100 mls / 1 ml sample = 100

100 x 17 = 1700 cfu/100 mls sample

Bacteria and



# Calculate average of triplicates

Easygel:

- Add number of mls of sample used
- Divide into 100 to get conversion factor
- Add number of colonies counted
- Multiply colonies by conversion factor



# Example - Easygel

Mls Used	Colonies counted	Calculated cfu/100 mls
1	10	1000
3	28	924
5	44	880

Average:

$$(1000+924+880) / 3 =$$

$$935 \text{ cfu/100 mls}$$

Correct calculation:

$$100 / 9 \text{ mls} = 11.11 \text{ conv.factor}$$

$$11.11 \times 82 = 911 \text{ cfu/100 mls}$$

# Calculate average of triplicates

Petrifilm:

- Simple average





# Enter data in data base

- [www.iwr.msu.edu/cmb](http://www.iwr.msu.edu/cmb)
- Password = ecoli (one word, lower case)
- Accepts all field data
- Will calculate triplicate averages
- Allows you to download your state data



# Wrap up

- Questions?
- Complete & return evaluation (green)
- Complete post-test (yellow)

THANKS for coming!

