

E. coli

Primary Importance:

Fecal coliform bacteria are found in the feces of warm-blooded animals, including humans, and are naturally present in the intestines of animals. *E. coli* is a type of fecal coliform bacteria that can lead to illness and indicate other pathogenic (disease-causing) bacteria in water. The presence of *E. coli* is an indicator of fecal contamination.

Problem

A high number of *E. coli* colonies indicate a potential problem for humans. Fecal coliforms are bacteria found in the feces of warm-blooded animals. While fecal coliforms themselves are not particularly harmful, the presence of large amount can indicate the presence of more harmful bacteria such as those that cause typhoid, cholera, and Hepatitis A. Ingestion of the other bacteria sometimes found with *E. coli* could lead to gastrointestinal distress and eye, nose and throat infections.

Causes

- ❖ Raw sewage dumped into rivers through Combined Sewer Overflows during heavy rains.
- ❖ Movement of feces to stream from animal farms or from animals utilizing streams or lakes for wading, drinking, or cooling.

Instructions:

The following instructions are adapted from those provided by Micrology Laboratories, Inc., for use with their patented Coliscan Easygel method. Please refer to the Color Guide identification sheets for interpretation of results.

CHECKLIST

- Pre-treated* petri dishes (only available from Micrology Laboratores)
- Sterile pipettes
- Whirl-pac bags or other sterile container
- Bottle of Coliscan Easygel
- Permanent marker
- Tape
- Rubber gloves
- Ice and cooler (if plating off-site)
- Bleach and water-tight bag for disposal
- Material Safety Data Sheets
- Testing Instructions
- Data Sheets

DO NOT rinse these materials before or after use. Follow instructions below.

1. Before you begin, label the top (lid) of the petri dish with a permanent marker. Include the date, time, location and volume (mL) of sample used. Also, remove Coliscan Easygel bottle from freezer and allow to defrost.

2. Wearing gloves and using only sterile equipment, obtain a sample for testing in one of two ways. Either collect your water sample in a sterile container (e.g. Whirl-pak Bag) and transport the water to an appropriate test site, or take a measured sample directly from the source using a sterile pipette and immediately place it into the bottle of Coliscan Easygel. In either case, obtain the sample slightly below the surface of the waterway you are testing. (Note: Water samples kept longer than 1 hour prior to plating, or any Coliscan Easygel bottle that has a sample placed into it for transport longer than 10 minutes, should be kept on ice or in a refrigerator.)
3. Transfer a measured amount of water from the sample container/source into the bottle of Coliscan Easygel. (*Note: For safety purposes and easier identification, the amount of sample transferred will vary according to the suspected condition of the surface water you are testing. If you suspect a high fecal coliform count due to contamination, transfer only 1 mL of sample. Typically, however, 3-5 mL is appropriate.*) Once the sample is transferred, swirl the bottle to distribute the Easygel mixture and then pour into the labeled petri dish. Being careful not to splash over the side or onto the lid, gently swirl and rock the filled dish until the mixture is evenly distributed.
4. While its contents are still in liquid form, place the dish right side up directly into a warm level spot indoors. **Solidification will occur in approximately 45 minutes.**
5. Turn the petri dish upside down (to reduce condensation) and incubate at 35°C for 24 hours or at room temperature for 48 hours.
6. **After the appropriate incubation period, inspect the dish. Count all of the purple colonies in the dish and record the results in terms of *E. coli* per 100 mL of water.** Disregard the light blue, blue-green, or white colonies. **DO NOT** count pinpoint colonies < 1 mm in size. To report the total number of *E.coli* per 100 mL, first divide 100 by the number of mL you used for your sample. Then multiply your count by that number.

EXAMPLE:

If a 3 ml sample displays 4 *E.coli* colonies, then first divide 100 by 3=33.3
Then multiply 33.3 x 4=**133.2 colonies/100mL**

7. To prepare your sample bottle and petri dish for disposal, place 5 mL (about 1 teaspoon) of straight bleach onto the surface of the medium. Allow mixture to sit for at least 5 minutes. Place in a watertight bag and discard in the trash.

TYPICAL RANGE FOR *E. coli* = 133 to 1,157 col/100 m

U.S. EPA Standards for <i>E. coli</i> in water (/100mL of sample)	
Alabama Water Watch: based on 1986 report (Ambient Water Quality Criteria for Bacteria, U.S. EPA, Washington, D.C., EPA 440/5-84-002)	
Piped Drinking Water	0
Drinking Water Source (pre-treatment)	2,000-4,000
Designated Beach Area	235
Moderate Swimming Area	298
Light Swimming Area	406
Rarely Used Swimming Area	576
If you wonder if you can swim in your area, contact your local health department.	

INTERPRETING COLISCAN® POUR PLATES

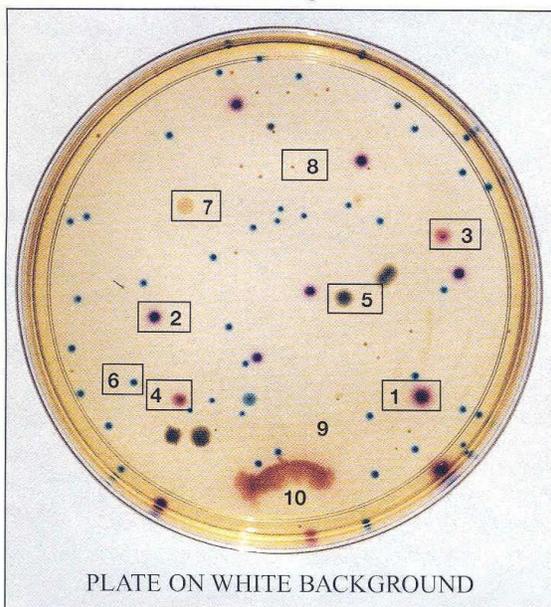
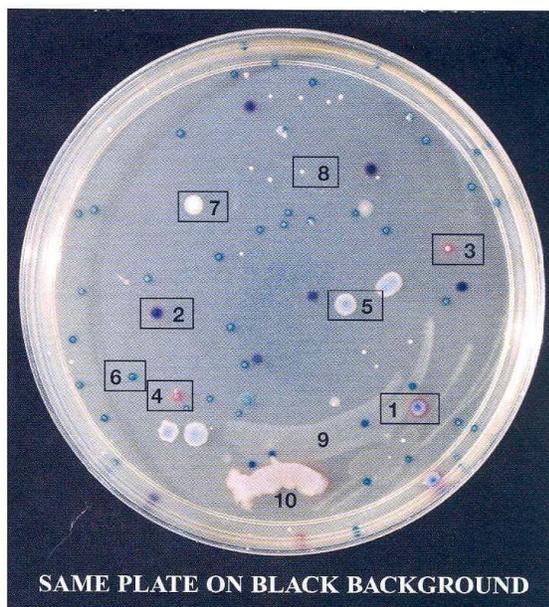


PLATE ON WHITE BACKGROUND



SAME PLATE ON BLACK BACKGROUND

Explanation of colony types (24-48 hrs. incubation)

- | | |
|---|-----------------------------------|
| 1. purple surface colony (hazy halo) | 6. blue-green submerged colony |
| 2. purple submerged colony | 7. white surface colony |
| 3. pink surface colony | 8. white submerged colony |
| 4. pink submerged | 9. white spreader on plate bottom |
| 5. blue-green surface colony (white halo) | 10. pink spreader on surface |

Note that submerged colonies are smaller than the same type growing on the exposed surface and color and appearance are different when viewed over different backgrounds.

No's. 1 & 2 are typical *E. coli* (fecal coliform) colonies which produce both galactosidase and glucuronidase and are purple due to the combination of the pink and blue-green chromagens that indicate the presence of the respective enzymes.

No's. 3 & 4 are typical general coliforms (Genera *Citrobacter*, *Enterobacter*, *Klebsiella*) which produce galactosidase and are therefore a pink colony color.

No's. 5 & 6 are characteristic of less common bacteria that produce glucuronidase only and are therefore a blue-green colony color.

No's. 7 & 8 are characteristic of bacteria that produce neither galactosidase nor glucuronidase and therefore are a white or colorless colony.

No's. 9 & 10 are spreaders and can each be counted as only one colony.

Bacteria that appear like No's. 5, 6, 7, 8 & 9 are likely members of the family Enterobacteriaceae, but are not technically coliforms because they don't produce the characteristic enzyme pattern. However, these types include such important genera as *Proteus*, *Salmonella* and *Shigella* and should not be ignored as insignificant.

MICROLOGY LABORATORIES, LLC., P.O. BOX 340, GOSHEN, IN 46526
PHONE: 219-533-3351 ■ FAX: 219-533-3370

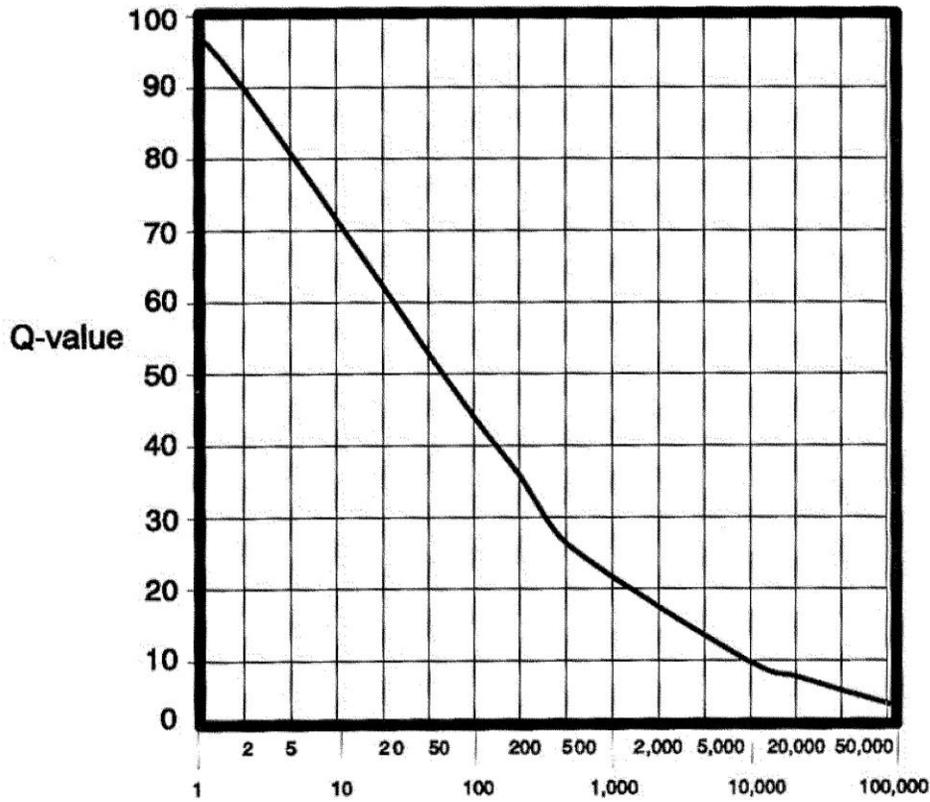
Interpreting the Plates

What to count as <i>E. coli</i>	What not to count as <i>E. coli</i>
 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 
Actual size of countable colonies = 1-2 mm.	$E. coli/100 \text{ ml} = \frac{(\# \text{ colonies counted} \times 100)}{\text{size of sample in ml}}$

Ecolichart.cdr
hp5500psdriver

Count dark blue and purple colonies only!

E. coli Q-values



FC: colonies/100 mL

Note: If FC > 10⁵, Q = 2.0

E. coli (colonies/100mL)	Q-value
0-1	98
2	89
5	80
10	71
20	63
50	53
100	45
200	37
500	27
1,000	22
2,000	18
5,000	13
10,000	10
20,000	8
50,000	5
100,000	3
>100,000	2