

# COLI-LAB



E.COLI TESTING EDUCATIONAL KIT



**STREAM**



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### **Educator Version**

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### Introduction

EBPI's Coli-Lab™ kit is a 96 well microplate kit which provides a rapid, convenient and accurate test for qualitative and quantitative measurement of the total Coliforms and E.coli bacteria. The Coli-Lab™ kit is designed to provide a hands-on experience on detecting bacterial indicators which is extremely important when assessing the microbiological safety of drinking and recreational waters.

### Purpose

The test is based on internationally regulated and approved methodology which is designed to meet the regulatory guidelines for untreated water, surface water, recreational water, processing water and wastewater. As the bacterial screening microplate contains all reagents required to indicate the presence of colony forming bacteria and E.coli, all that is required is sample waters and incubation of the Coli-Lab™.

The Coli-Lab™ kit is based on the unique ability of Coliform bacteria to utilize specialized nutrients and reagents to form a distinctive blue/green colour, and the unique ability of E.coli to form a fluorescent appearance under the assay conditions. The kit can also provide a quantitative analysis of Coliforms and E.coli from the principle of the Most Probable Number (MPN) of colony forming-units per 100 mL.

### WARRANTY

EBPI warrants that, at the time of shipment, the Coli-Lab™ kit is free of defects in material and workmanship, and complies with the company specifications. Since actual experimental conditions prevailing at user's laboratory are beyond the control of EBPI or its representatives, EBPI makes no other warranty, express or implied, with respect to the product. Notification of any breach of warranty must be made within 120 days of delivery. The sole and exclusive remedy of the customer for any liability of EBPI of any kind, including liability based upon warranty (express or implied, whether contained herein or elsewhere) is limited to the replacement of the product or the refund of the invoice price of the product.

### Concept: E.coli and Coliform

Bacteriological water quality guidelines provide mandatory limits for Total Coliform and E.coli in recreational waters without further specifying the methods to be used. As a result there is often an inconsistency in methods used to enumerate indicator bacteria in recreational water quality monitoring initiatives. Three methods, membrane filtration (MF), multiple tube fermentation (MTF), and defined substrate technology (DST). The three methods are each based upon measuring different products of bacterial growth. MF enumerates bacterial colonies on a specific growth substrate. MTF measures metabolic activity as determined by fermentation and the production of gas.

Defined substrate technology (DST) methods on the other hand, including the Coli-Lab™ technique are a more reliable technique than the standard methods for enumerating bacteria in natural waters. It is also recognized as appropriate procedures to enumerate indicator bacteria for routine surface water quality monitoring from the American Public Health Association. DST methods measure the ability of organisms to metabolize a specific labelled substrate, thereby releasing a chromogen (pigment-producing enzyme).

The rapid detection of bacterial indicators is extremely important when assessing the microbiological safety of drinking and recreational waters. The introduction of rapid defined substrate techniques, which utilize chromogenic and fluorogenic substrates incorporated into selective media has led not only to the faster detection of bacterial indicators but also the improved accuracy for their enumeration.

## E.coli

E.coli is a type of coliform bacteria and is found in the intestines of warm-blooded animals, including humans. Most bacterial strains of E.coli are not harmful to humans and in fact help digest essential nutrients in our digestive systems. Some strains of E.coli bacteria however can cause severe cramps, diarrhea and in extreme cases, even death, as what occurred in Walkerton, Ontario, Canada in 2000 with the O157:H7 strain.

## Coliform

Coliform bacteria occur naturally in the environment and are not harmful themselves. Their presence in water does however suggest that other disease-causing organisms may be present. These bacteria have been selected as an indicator in many nations, with their presence indicating problems with water treatment or distribution.

## **The Reaction**

The Coli-Lab utilizes proven nutrient indicators X-Gal and MUG to detect viable Coliforms and E.coli bacteria as well as selective media to stimulate the growth and indicators for chromogenic and fluorogenic enzymes. The substrates react with the enzymes produced by the Coliforms (Beta-D-galactosidase) and the E.coli (Beta-D-glucuronidase) resulting in a blue/green formation of colour, and a fluorescence emission by the Coliforms and E.coli respectively. The presence of any strain of E.coli in waters is a strong indication of recent sewage or animal water contamination. As with coliforms, the presence of any strain of E.coli indicates other harmful strains or other organisms could be present and could pose a threat to human health. Coliform positive test results in a distinctive blue colour, which enables analysis of brownish, turbid or rust-filled water.

The presence of any strain of E.coli in waters is a strong indication of recent sewage or animal water contamination. As with coliforms, the presence of any strain of E.coli indicates other harmful strains or other organisms could be present and could pose a threat to human health. Coliform positive test results in a distinctive blue colour, which enables analysis of brownish, turbid or rust-filled water.



**Expectations**

The Coli-Lab™ kit is designed to provide a hands-on experience on detecting bacterial indicators which is extremely important when assessing the microbiological safety of drinking and recreational waters. Please note that this experiment was designed to work in groups of three to four. Each group will be given one 96-well microplate to complete their lab study and analysis of the results. Students in their groups will submit a lab report for your evaluation.

**Contents of the Coli-Lab™ Kit**

Each kit contains 8x 96-well microplates to complete the experiment.	You will require the following to complete this experiment (contact EBPI for further details):		
	Longwave UV light	Glasses for using the UV light	Incubator
	1x Ziploc bag (medium sized)	Multichannel or Single Pipettes	

**Collecting the Samples**

You will have the option of either having your students acquire samples from pre-determined or assigned sites that you and/or your class discussed or you can acquire the samples. The samples can be acquired from any site with a body of water. Ensure your students understand and follow safety measures when acquiring the samples. Each group will require 30mL to 60mL (approximately 1 to 2 oz). The class should have a total of 6 samples organized as follows:

- |            |            |            |            |            |            |
|------------|------------|------------|------------|------------|------------|
| Group 1    | Group 2    | Group 3    | Group 4    | Group 5    | Group 6    |
| - Sample 1 | - Sample 2 | - Sample 3 | - Sample 4 | - Sample 5 | - Sample 6 |

**Preparation of the Samples**

There is no advance preparation required for the samples collected. Ensure that the samples are kept in room temperature and they are sealed in the containers the samples were collected.

**Student Station Preparation**

Below is the suggested distribution of supplies for the preparation of the lab stations for the day of the experiment:	<u>FOR EACH STUDENT LAB STATION</u>	<u>FRONT OF THE CLASSROOM</u>
	<ul style="list-style-type: none"> <li>- 1x 96-well microplate</li> <li>- Multichannel or Single Pipette</li> <li>- Their test sample (30mL to 60mL)</li> </ul>	<ul style="list-style-type: none"> <li>- Longwave UV light</li> <li>- Glasses for using the UV light</li> <li>- Incubator</li> <li>- Ziploc bag (medium sized)</li> </ul>

**Lab Safety Protocols – Review this with your class before beginning the experiment**

Storage:

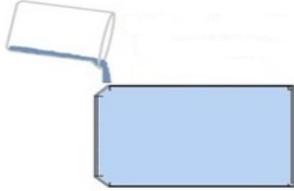
- All kit components should be stored in the sealed packaging provided.
- Ensure the storage area is dry and in temperatures ranging from 2°C to 30°C.
- Keep the kit away from light.
- In humid climates, it is recommended that Coli-Lab™ are stored in a refrigerator.

Handling:

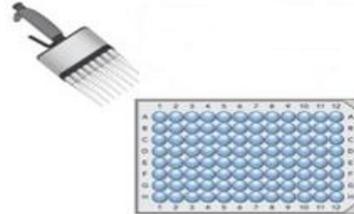
- Though no bacteria is include in the kit, other bacteria and other living organisms may be contained in the water samples that are collected.
- The test is designed to support the growth of microorganisms in the sample waters; this may include pathogens.
- Hands should be thoroughly washed after handling exposed Coli-Lab™.



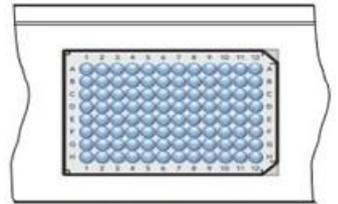
- 1.** Pour water sample (20-30 mL) into the microplate lid.



- 2.** Using a multichannel pipette (if available), dispense 200 uL of water to each well of the microplate. If there is no multichannel pipette, use a dropper or slowly pour the sample and fill it to the top. Check for air bubbles by gently tapping the side.



- 3.** Discard the remaining water from the lid. Use the same lid to cover the microplate and place the microplate into a Ziploc bag.

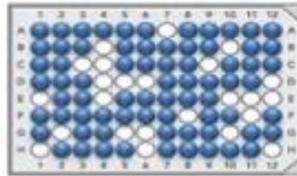


- 4.** Incubate at 35°C for 24 hours. If there is no incubator, place the Coli-Lab in a warm location away from direct sunlight.

Below are the incubation times based on temperature:

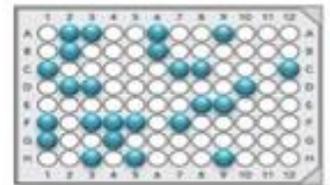
- \* 20°C to 25°C, incubate for 60 hours
- \* 25°C to 30°C, incubate for 48 hours
- \* 30°C to 35°C, incubate for 36 hours
- \* 35°C to 37°C, incubate for 24 hours

- 5.** Count the number of blue wells and refer to the MPN table to determine the Most Probable Number of Total Coliforms in 100 mL of water.



- 6.** Observe the microplate under long UV (366 nm) light.

- 7.** Count the number that are both blue and fluorescent under the UV light and refer to the MPN table to determine the Most Probable Number of total *E. coli* in 100 mL of water.



- 8.** Do not forget to clean up once the experiment is done. Used microplates should be soaked for a minimum of 24 hours in a bleach water solution to ensure all micro-organisms are killed prior to the plastics being recycled. Remember that proper disposal is essential.



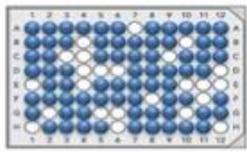
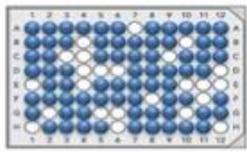
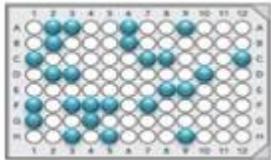
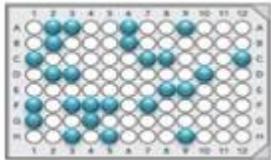
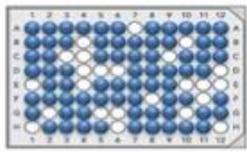
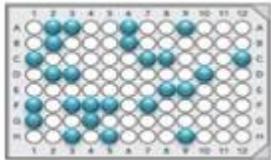
**MPN Table: Most Probable Number of Coliform and *E.coli* per 100 mL**

Number of Wells with a Positive Reaction	MPN 100 mL Sample	Number of Wells with a Positive Reaction	MPN 100 mL Sample	Number of Wells with a Positive Reaction	MPN 100 mL Sample
0	< 3	33	106	65	298
1	3	34	110	66	307
2	5	35	114	67	317
3	8	36	119	68	328
4	11	37	123	69	339
5	13	38	127	70	350
6	16	39	132	71	362
7	19	40	136	72	375
8	22	41	141	73	388
9	25	42	146	74	403
10	28	43	151	75	418
11	30	44	156	76	443
12	33	45	161	77	451
13	36	46	166	78	469
14	39	47	171	79	489
15	43	48	177	80	510
16	46	49	182	81	534
17	49	50	188	82	559
18	52	51	194	83	587
19	55	52	200	84	619
20	59	53	206	85	654
21	62	54	213	86	694
22	65	55	219	87	740
23	69	56	226	88	794
24	72	57	233	89	858
25	76	58	240	90	938
26	79	59	247	91	1038
27	83	60	255	92	1174
28	87	61	263	93	1370
29	90	62	271	94	1696
30	94	63	280	95	2424
31	98	64	289	96	>2424
32	102				



To Educators: Please review the information as a visual guideline of the experiment results, recording the results, further analysis, and the answer sheet for the questions.

**Recording the Results** – There are two parts where students need to record results.

Part 1: Counting the total Coliforms (Steps 4 and 5)	Part 2: Counting the total <i>E.coli</i> (Steps 6 and 7)				
<p>The microplate would be incubated overnight at 35°C to 37°C (or refer to the incubation chart – see Procedure). Students would then place the microplate on a white surface.</p> <table border="1" data-bbox="110 716 795 976"> <tr> <td data-bbox="110 716 506 976"> <p>When students are completing Step 5, it is important for accuracy that students count ALL of the blue wells regardless of the intensity of the colour. This represents a positive reaction for Coliforms.</p> </td> <td data-bbox="506 716 795 976">  </td> </tr> </table> <p>Refer to the MPN table to determine the Most Probable Number of Coliform and <i>E.coli</i> forming units in sample water.</p>	<p>When students are completing Step 5, it is important for accuracy that students count ALL of the blue wells regardless of the intensity of the colour. This represents a positive reaction for Coliforms.</p>		<p>After the completion of Step 5, students will place the incubated microplate on a black (dark) surface in reduced light and observe with glasses under long wavelength UV (366 nm) light.</p> <table border="1" data-bbox="824 716 1510 1010"> <tr> <td data-bbox="824 716 1198 1010"> <p>When they are completing Step 7, it is important for accuracy that students count ONLY the wells that turned blue AND were fluorescent under the UV light. This represents a positive reaction for <i>E.coli</i>.</p> </td> <td data-bbox="1198 716 1510 1010">  </td> </tr> </table> <p>Do NOT count the wells that are NOT both blue and fluorescent. Refer to the MPN table to determine the Most Probable Number of Coliform and <i>E.coli</i> forming units in sample water.</p>	<p>When they are completing Step 7, it is important for accuracy that students count ONLY the wells that turned blue AND were fluorescent under the UV light. This represents a positive reaction for <i>E.coli</i>.</p>	
<p>When students are completing Step 5, it is important for accuracy that students count ALL of the blue wells regardless of the intensity of the colour. This represents a positive reaction for Coliforms.</p>					
<p>When they are completing Step 7, it is important for accuracy that students count ONLY the wells that turned blue AND were fluorescent under the UV light. This represents a positive reaction for <i>E.coli</i>.</p>					

During this time, you can also assess and evaluate their results to note accuracy and any possible errors. Students should be encouraged to record their errors in their lab reports.

Students would then record the results in the chart below:

Group (Microplate) Number	Water Type / Location	Positive Reaction for Total Coliforms	Positive Reaction for Total <i>E.coli</i>
1			
2			
3			
4			
5			
6			
7			
8			

## Further Analysis

You can pick further analysis questions based on your preference and what you want your students to learn.

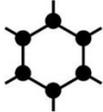
### Questions and Answers

- The Coli-Lab™ water testing kit is a 96 well microplate kit which provides a rapid, convenient and accurate test for qualitative and quantitative measurement of the total Coliforms and E. coli bacteria. The test is based on internationally regulated and approved methodology which is designed to meet the regulatory guidelines for untreated water, surface water, recreational water, processing water and wastewater. As the bacterial screening microplate contains all reagents required to indicate the presence of colony forming bacteria and E.coli, all that is required is sample waters and incubation of the Coli-Lab™. The substrates react with the enzymes produced by the Coliforms (Beta-D-galactosidase) and the E. coli (Beta-D-gluconidase) resulting in a blue/green formation of colour, and a fluorescence emission by the Coliforms and E. coli respectively.
1. Describe in your own words what the Coli-Lab™ is and how it works. What are the benefits of using these kits? What kind of technique is used in the Coli-Lab™?
- The benefits of using Coli-Lab™ include the following:
- ideal for offsite field sampling and you do not require laboratory conditions or confirmatory testing
  - it is cost effective alternative to the traditional standard methods
  - faster turnaround of results (1-3 days)
  - user-friendly and efficient
  - enables the results to be reported in a timely manner
  - improves water quality monitoring initiatives to be effectively undertaken regardless of the location
2. Provide a brief description of the locations chosen and compare the results above. Which location had the highest MPN? The lowest? What is the significance of this in relation to the number of E.coli and Coliforms?
- Defined substrate technology (DST) methods measure the ability of organisms to metabolize a specific labelled substrate, thereby releasing a colour producing enzyme.
- It will vary depending on what locations and/or water samples you use, but in terms of significance, the higher the number of E.coli and Coliforms, the more harmful the water will be. The presence of any strain of E.coli indicates other harmful strains or other organisms could be present and could pose a threat to human health.
3. Besides testing drinking water, briefly describe one application that you think you can use the Coli-Lab™ for.
- A number of applications can be used such as ...
- Safety of swimming pools or public water spaces
  - Public water fountains
  - Presence of contaminants in natural habitats or watersheds
  - Analysis of hydrological systems (i.e. surface water, groundwater, waste water, and distribution systems)
  - Sources or degree of bacterial contamination which exists in our environment
  - and so on ...
4. Steps 6 and 7 indicate that only the wells that are blue and are fluorescent should be counted, what would be the reason for this?
- The Coli-Lab™ contains nutrient indicators (X-Gal and MUG) to detect viable Coliforms and E.coli bacteria to stimulate the growth and indicators for chromogenic and fluorogenic enzymes. The substrates react with the enzymes produced by the Coliforms (Beta-D-galactosidase) and E.coli (Beta-D-gluconidase) resulting in blue/green colour, and a fluorescence by the Coliforms and E.coli respectively.



Group #	Group Student Names	Role

**Checkmark the appropriate Stream below:**

<b>Water</b> 	<b>Chemical</b> 	<b>Soil</b> 	<b>Synthetic</b> 

**Note:** At the beginning of class, your teacher will have the lab stations ready. Remember to check your lab station, ensure you have the appropriate supplies, and read over the safety protocols.

<b>Pre-Lab Notes</b>	
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**Instructions:** Read over the preparation and the procedure for the specific biotechnology kit that you will be working on. Complete the lab and record the results.



## Preparation – Coli-Lab™



To Students: Please follow the instructions and information below to help prepare for the lab experiment.

### Expectations

The Coli-Lab™ kit is designed to provide a hands-on experience on detecting bacterial indicators which is extremely important when assessing the microbiological safety of drinking and recreational waters. You will work in groups of three to four with one 96-well microplate per group unless otherwise indicated by your educator. Your group will follow the procedure to complete the lab experiment, record the results, complete your lab analysis, and submit the report to your educator for evaluation.

### Collecting the Samples

If you are completing the Site Sample Study, your group will need to collect a sample from your assigned or pre-discussed site. Your water sample will be approximately 30mL to 60mL (approximately 1 to 2 oz.). This sample will be the one your group will work on. If your educator collected the samples, your group will be assigned one of these samples to work on.

The class will have a total of 6 samples organized as follows:

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
- Sample 1	- Sample 2	- Sample 3	- Sample 4	- Sample 5	- Sample 6

### Preparation of the Samples

There is no preparation required for the samples collected. Ensure that the samples are kept in room temperature and they are sealed in the containers the samples were collected.

### Lab Station Preparation

#### FRONT OF THE CLASSROOM

#### GROUP LAB STATION

At the beginning of the lab, you should have the following available in the front of the classroom and lab station:

- Longwave UV light
- Glasses for using the UV light
- Incubator
- Ziploc bag (medium sized)

- 1x 96-well microplate
- Multichannel or Single Pipette
- Their test sample (30mL to 50mL)

Before starting an experiment it is important to ensure all of the materials, supplies and equipment needed are available and ready. Be sure to speak to your educator if there is anything missing.

### Ensure that you review the Lab Safety Protocols before beginning the experiment:

#### Storage:

- All kit components should be stored in the sealed packaging provided.
- Ensure the storage area is dry and in temperatures ranging from 2°C to 30°C.
- Keep the kit away from light.
- In humid climates, it is recommended that Coli-Lab™ are stored in a refrigerator.

#### Handling:

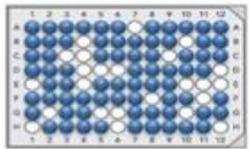
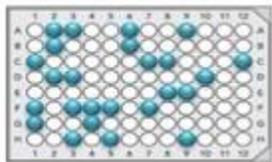
- Though no bacteria is included in the kit, other bacteria and other living organisms may be contained in the water samples that are collected.
- The test is designed to support the growth of microorganisms in the sample waters; this may include pathogens.
- Hands should be thoroughly washed after handling exposed Coli-Lab™.



**Instructions:** Once you obtained the results, complete the following steps below. Read over the guidelines before recording your results.

If you have any errors from the experiment, please describe them here and the reasons why this occurred:

**Observing the Results** – There are two parts to review below when you are recording your results.

<p><b>Part 1: Counting the total Coliforms</b> (Steps 4 and 5)</p>	<p><b>Part 2: Counting the total <i>E.coli</i></b> (Steps 6 and 7)</p>
<p>The microplate would be incubated overnight at 35°C to 37°C (or refer to the incubation chart – see Procedure). Place the microplate on a white surface.</p> <div data-bbox="110 1081 495 1302" style="border: 1px solid black; padding: 5px; width: fit-content;"> <p>When you are completing Step 5, it is important for accuracy that you count ALL of the blue wells regardless of the intensity of the colour. This represents a positive reaction for Coliforms.</p> </div> <div data-bbox="527 1117 776 1266" style="text-align: center;">  </div> <p>Refer to the MPN table (found with the procedure) to determine the Most Probable Number of Coliform and <i>E.coli</i> forming units in sample water.</p>	<p>After the completion of Step 5, you will place the incubated microplate on a black (dark) surface in reduced light and observe with glasses under long wavelength UV (366 nm) light.</p> <div data-bbox="831 1117 1185 1411" style="border: 1px solid black; padding: 5px; width: fit-content;"> <p>When they are completing Step 7, it is important for accuracy that you count ONLY the wells that turned blue AND were fluorescent under the UV light. This represents a positive reaction for <i>E.coli</i>.</p> </div> <div data-bbox="1218 1182 1490 1344" style="text-align: center;">  </div> <p>Do NOT count the wells that are NOT both blue and fluorescent. Refer to the MPN table (found with the procedure) to determine the Most Probable Number of Coliform and <i>E.coli</i> forming units in sample water.</p>

**Recording the Results** – Table of Positive Reactions for total Coliforms and *E.coli*:

Record your results on the table below and record the class results as well.

<b>Group (Microplate) Number</b>	<b>Water Type / Location</b>	<b>Positive Reaction for Total Coliforms</b>	<b>Positive Reaction for Total <i>E.coli</i></b>
<b>1</b>			
<b>2</b>			
<b>3</b>			
<b>4</b>			
<b>5</b>			
<b>6</b>			
<b>7</b>			
<b>8</b>			

**Further Analysis** – Please complete the following questions below.

Describe in your own words what the Coli-Lab™ is and how it works. What are the benefits of using these kits? What kind of technique is used in the Coli-Lab™?

Provide a brief description of the locations chosen and compare the results above. Which location had the highest MPN? The lowest? What is the significance of this in relation to the number of E.coli and Coliforms?

Besides testing drinking water, briefly describe one application that you think you can use the Coli-Lab™ for.

Steps 6 and 7 indicate that only the wells that are blue and are fluorescent should be counted, what would be the reason for this?