



## Aquagenx® CBT EC+TC (Compartment Bag Test) Most Probable Number (MPN) Kit Instructions for Use: 1:10 Dilution for Surface and Recreational Waters

### Overview

The Aquagenx CBT EC+TC MPN Kit simultaneously detects and quantifies *E. coli* (EC) and Total Coliform (TC) bacteria in a 100 mL sample. It uses a proprietary powder growth medium with a glucose substrate called X-Gluc. When *E. coli* metabolize this substrate in Aquagenx's growth medium, the color of the water turns blue, indicating the presence of *E. coli*. The growth medium also contains a fluorogenic galactoside substrate called MUGal. If total coliforms are present, they metabolize this fluorogenic substrate and the sample fluoresces blue under a UV light (365 nm). Most Probable Number (MPN) test results are obtained by easy color match using the Aquagenx color-coded MPN Table. The total coliform group of bacteria includes *E. coli*, which is a fecal coliform as well as a thermotolerant coliform. Not all total coliforms are *E. coli*.

**Dilutions Guidance and Wastewater Dilutions:** <https://www.aquagenx.com/dilutions-cbt-ectc/>

**Product documents:** <https://www.aquagenx.com/product-documentation/>

### Shelf Life of Growth Medium

Aquagenx® EC+TC powder growth medium is stable up to two-years after date of manufacture at 25° Celsius. Expiration date and lot number are printed on back of medium packet.

### Storage of Growth Medium

Recommended storage temperature is 10-25° Celsius. Growth medium can be stored in a refrigerator. Cold chain for Aquagenx EC+TC growth medium is not required.

### Summary of Test Procedures for CBT EC+TC MPN Kit 1:10 Dilution

<p>Collect 10 mL sample, add 90 mL sterile water</p>	<p>Add powder growth medium</p>	<p>Pour sample into compartment bag</p>	<p>Roll down Whirl-Pak seal and attach plastic clip</p>
<p>Incubate 20-48 hours</p>	<p>Score MPN test results for EC under ambient light</p>	<p>Score MPN test results for TC under UV light</p>	<p>Decontaminate sample</p>

### How to Interpret Color-Change Test Results

Color of compartment in Compartment Bag	Yellow/Yellow Brown in ambient light and does not fluoresce blue under UV light	Yellow/Yellow Brown that ... fluoresces blue under UV light	Blue/Blue Green in ambient light	Blue/Blue Green that... fluoresces blue under UV light
	<i>E. coli</i>	Negative	Negative	Positive
Total Coliforms	Negative	Positive	Positive	Positive

## Basis of Aquagenx CBT Most Probable Number (MPN) Table

The Aquagenx CBT MPN Table originally was designed for drinking water testing and based on the World Health Organization "Guidelines for Drinking Water Quality," 4th Edition. We modified the MPN Table on page 4 to reflect a 1:10 dilution, which increases the CBT's range of detectable bacteria by a factor of 10. MPN of *E. coli* per 100 mL is estimated from the combination of positive (blue color) and negative (no blue color) compartments in the Aquagenx Compartment Bag. MPN of total coliforms per 100 mL is estimated from the combination of positive (blue fluorescence under UV light) and negative (no blue fluorescence under UV light) compartments in the Aquagenx Compartment Bag.

## U.S. EPA 2012 Recreational Water Quality Criteria

Aquagenx MPN Table risk categories are based on EPA Recommendation 1 for *E. coli*

CRITERIA ELEMENTS	Recommendation 1 Estimated Illness Rate 36/1,000		Recommendation 2 Estimated Illness Rate 32/1,000	
	GM (cfu/100 mL)	STV (cfu/100 mL)	GM (cfu/100 mL)	STV (cfu/100 mL)
Enterococci (marine and fresh)	35	130	30	110
<i>E. coli</i> (fresh)	126	410	100	320

Source: U.S. EPA Office of Water, EPA-820-F-12-061, Recreational Water Quality Criteria Fact Sheet

**PROCEDURAL NOTES. SEE HOW-TO VIDEOS:** <https://www.aquagenx.com/how-to-use-cbt-ectc/>

### 1. Prepare work area

- Sanitize work area with disinfectant cleaning solution, paper towels or wipes.

### 2. Collect 10 mL water sample to add to Whirl-Pak® Thio-Bag®

- Wearing disposable, thin plastic gloves is recommended. If you don't have gloves, do not touch inside of Thio-Bag with bare hands.
- White tablet in Thio-Bag is sodium thiosulfate, which neutralizes residual chlorine if present in sample. Do not remove it from the bag.
- Fill a sterile 10 mL pipette with water sample and pour into Thio-Bag.

### 3. Add 90 mL of diluent to Whirl-Pak Thio-Bag

- Add 90 mL of *E. coli*-free water to Thio-Bag (up to 100 mL fill line).
- Diluent options: Standard Methods phosphate buffer; Autoclaved or boiled reagent water; Autoclaved or boiled tap water; High quality, certified bottled water. Some bottled waters are produced locally and are microbiologically unsafe; Distilled water from a commercial source and sold in properly sealed bottles. While not necessarily sterile, it should be free of *E. coli*.
- Record sample details such as date, time and location.

### 4. Add Aquagenx EC+TC growth medium to sample in Whirl-Pak Thio-Bag

- We recommend testing procedure begins within six hours of sample collection. Do not add growth medium to the Thio-Bag until you are ready to complete the entire testing procedure.
- Open growth medium packet. Tear downward on serrated edge on medium packet that is nearest to letters EXP.
- Pour powder growth medium into Thio-Bag. Do not touch growth medium with bare fingers or hands.
- Roll down Whirl-Pak seal and close Thio-Bag shut.
- Dissolve medium in sample. Gently swirl the bag and squeeze clumps of powder until medium is dissolved.

### 5. Pour sample with dissolved medium from Thio-Bag into Aquagenx Compartment Bag

- Label Compartment Bag or attach barcode asset tag to Compartment Bag. Tear off perforated seam at top of bag.
- Rub top of bag and sides of bag together to open so sample can run into each compartment.
- Use white tabs at top of Compartment Bag to pull bag open. Do not touch inside of bag with bare fingers or hands.
- Slowly pour sample into bag while gently tilting and squeezing bag to distribute sample among five compartments.
- Fill evenly to the top of the fill line across all five compartments.

## 6. Seal Compartment Bag shut

- Roll down Whirl-Pak seal at top of Compartment Bag and fasten shut.
- Attach plastic seal clip across Compartment Bag to prevent water from leaking out of compartments. Place U-shape part of clip across width of bag along the fill line and *below the compartment openings*. Place rod-shaped part of the clip on the opposite side of Compartment Bag and snap into U-shape to lock in place.

## 7. Incubation Period and Temperatures

- During the incubation period, CBTs can develop an odor. To help control odor, place CBTs in another sealed plastic bag or container during the incubation period.
- Ambient temperature incubation works at any temperatures between 25°- 44.5°C for detection of *E. coli* and/or total coliforms.
- Because the CBT works at variable temperatures, constant temperature control in an incubator is not required. However, at cooler temperatures, constant temperature incubation is recommended, if available.
- Note: over 40°C, some total coliforms will be inhibited, and the results may not be accurate for total coliform analysis.
- For regulatory compliance purposes, samples must be incubated at 35-37°C for 20-24 hours to detect and quantify *E. coli* and total coliforms.
- The CBT also can be used to detect and quantify thermotolerant (fecal) coliforms instead of total coliforms if the CBT samples are incubated at a temperature of 44.5°C (between 44-45°C) throughout an incubation period of 20-24 hours. Strict temperature control is required for this procedure.

### Recommended Incubation Periods at Ambient Temperature Conditions:

35-37°C: Incubate 20 hours  
31-34°C: Incubate 24-30 hours  
25-30°C: Incubate 40-48 hours

Below 25 C: Incubate in a portable incubator at 35-37°C for 24 hours or put in or near another heat source for up to 48 hours, depending on the temperature.

Over 40°C: Some total coliforms will be inhibited, and the results may not be accurate for total coliforms.

See “Incubation Period Guidance”: <https://www.aquagenx.com/product-documentation/>

## 8. Score MPN test results

- Hold the Compartment Bag next to Aquagenx MPN Table on page 4 to score test results.
- ***E. coli* – view in ambient light:**
  - Yellow/yellow-brown compartment is negative for *E. coli* (absence).
  - Blue/blue-green compartment is positive for *E. coli* (presence). Positive compartments include any trace of blue/blue-green, such as one or more specks of blue/blue-green, or blue/blue-green sediment at bottom of a compartment.
- **Total Coliform - shine UV light (365 nm) on Compartment Bag in dark environment:**
  - Compartments that fluoresce blue are positive for total coliforms. These include any compartments that are yellow/yellow-brown in ambient light that fluoresce blue under UV light.
  - Compartments that are blue/blue-green in ambient light (positive for *E. coli*) are by definition also positive for total coliforms.
- Match color sequence of all five compartments to one of 32 color-coded rows in MPN Table to obtain MPN test results for *E. coli* and total coliforms.
- Record test results.

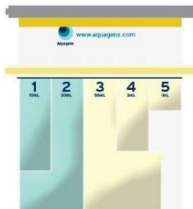
## 9. Decontaminate sample

- Add 4 mL of liquid bleach (NaOCl) or sufficient chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) to compartment bag to provide about 200 milligrams of free chlorine.
- After 30 minutes, pour contents into a sink, toilet or hole in ground and safely dispose the empty compartment bag.
- Retain plastic seal clip for reuse.

## Aquagenx® CBT Most Probable Number (MPN) Table Adapted for 1:10 Dilution

Align the Compartment Bag so compartment #1 is on the left and compartment #5 is on the right. Match the color sequence of all five compartments to one of the 32 color-coded rows. Each compartment is scored according to the following criteria (also see color chart on page 1):

- Yellow compartment with and without UV light exposure is negative for *E. coli* and total coliforms
- Yellow compartment with blue fluorescence under UV light is positive for total coliforms
- Blue compartment in ambient light is positive for *E. coli* and by definition also is positive for total coliforms



Row Number:	Compartment Number					MPN/100mL	Upper 95% Confidence Level/100mL	U.S. EPA Recreational Water Health Risk Category Based on MPN and Upper 95% Confidence Level
	1 10mL	2 30mL	3 56mL	4 3mL	5 1mL			
1	Yellow	Yellow	Yellow	Yellow	Yellow	0	28.70	Low Risk/Safe
2	Yellow	Yellow	Yellow	Blue	Yellow	10	51.40	Low Risk/ Probably Safe
3	Yellow	Yellow	Yellow	Yellow	Blue	10	47.40	
4	Blue	Yellow	Yellow	Yellow	Yellow	11	51.60	
5	Yellow	Blue	Yellow	Yellow	Yellow	12	56.40	
6	Yellow	Yellow	Blue	Yellow	Yellow	15	78.10	
7	Yellow	Yellow	Yellow	Blue	Blue	20	63.20	
8	Blue	Yellow	Yellow	Blue	Yellow	21	68.50	
9	Blue	Yellow	Yellow	Yellow	Blue	21	66.40	
10	Yellow	Blue	Yellow	Blue	Yellow	24	78.10	
11	Yellow	Blue	Yellow	Yellow	Blue	24	81.20	
12	Blue	Blue	Yellow	Yellow	Yellow	26	85.10	
13	Blue	Yellow	Yellow	Blue	Blue	32	83.80	
14	Yellow	Blue	Blue	Blue	Blue	37	97.00	
15	Yellow	Yellow	Blue	Yellow	Blue	31	113.60	
16	Yellow	Yellow	Blue	Blue	Yellow	32	118.20	
17	Blue	Yellow	Blue	Yellow	Yellow	34	125.30	
18	Blue	Blue	Yellow	Yellow	Blue	39	104.30	
19	Blue	Yellow	Yellow	Blue	Yellow	40	109.40	
20	Yellow	Blue	Blue	Yellow	Yellow	47	227.50	
21	Yellow	Yellow	Blue	Blue	Blue	52	147.30	
22	Blue	Blue	Yellow	Blue	Yellow	54	129.30	
23	Blue	Yellow	Blue	Yellow	Blue	56	171.40	
24	Blue	Yellow	Blue	Blue	Yellow	58	168.70	
25	Blue	Yellow	Blue	Blue	Blue	84	211.90	
26	Yellow	Blue	Blue	Yellow	Blue	91	370.40	
27	Yellow	Blue	Blue	Blue	Yellow	96	376.80	
28	Blue	Blue	Blue	Yellow	Blue	136	830.60	
29	Yellow	Blue	Blue	Blue	Blue	171	563.50	
30	Blue	Blue	Blue	Yellow	Blue	326	1455.50	
31	Blue	Blue	Blue	Blue	Yellow	483	3519.10	
32	Blue	Blue	Blue	Blue	Blue	>1000	94351.00	Very Unsafe

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