



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

Overview

The Aquagenx® CBT EC+TC MPN Kit simultaneously detects and quantifies *E. coli* (EC) and Total Coliforms (TC) 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 (365nm). 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*.

Shelf Life

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

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

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>

Note: For 1:100 and 1:1000 dilutions, see: <https://www.aquagenx.com/dilutions-cbt-ectc/>

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 (page four) is based on the World Health Organization “Guidelines for Drinking Water Quality,” 4th Edition.

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

Note: 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

1. Prepare work area

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

2. Collect 10 mL water sample with Whirl-Pak™ Thio-Bag™

- White tablet in Whirl-Pak™ Thio-Bag™ is sodium thiosulfate, which neutralizes residual chlorine in sample. Do not remove.
- Wearing disposable, thin plastic gloves is recommended. If you don't have gloves, avoid touching inside of Thio-Bag with bare hands.
- Fill a 10 mL pipette with water sample and pour into Thio-Bag.
- Add 90 mL sterile water to Thio-Bag (up to 100 mL fill line). Record sample details.

3. Add Aquagenx® EC+TC growth medium to sample in Whirl-Pak™ Thio-Bag™

- Open growth medium packet with scissors and pour powder growth medium into Thio-Bag. Growth medium should not be added to sample before you are ready to begin test procedure.
- 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 until the medium is completely dissolved. You can squeeze any clumps of powder to help them dissolve more quickly.

4. 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 and sides of bag together in each compartment to open so water easily runs into compartments.
- Use white tabs at top of bag to pull compartment bag open.

- 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.

5. 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 compartment bag along the fill line but *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.

6. Incubation Period and Temperatures

- During the incubation period, CBTs can develop an odor. To 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 (or fecal) coliforms instead of total coliforms, if the CBT bags 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 coliforms will be inhibited, and the results may not be accurate for total coliforms.

7. Score and record MPN test results

- Align compartment bag in correct sequence to Aquagenx® MPN Table on page four, hold bag up to read results.
- ***E. coli*:**
 - Yellow/yellow-brown indicates negative (absence) compartment for *E. coli*.
 - Blue/blue-green indicates positive (presence) compartment for *E. coli*. These include:
 - Any trace of blue/blue-green, or just specks of blue/blue-green, or just blue/blue-green sediment at bottom of compartment is considered a positive compartment.
- **Total Coliforms - shine UV light (365 nm) on compartment bag in a dark environment:**
 - Compartments that fluoresce blue are positive for total coliforms. These include:
 - Any compartments that are yellow/yellow brown in color that fluoresce blue under UV light.
 - Any compartments that are blue/blue-green in color are positive for *E. coli* are by definition positive for total coliforms.
- Match color sequence of 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.
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8. Decontaminate sample

- Add 4 mL of liquid bleach (NaOCl) or sufficient chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) 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

The Aquagenx® MPN Table is adapted for measuring *E. coli* and Total Coliforms in a 1:10 dilution with the Aquagenx® Compartment Bag Test. Risk categories are based on U.S. EPA Recreational Water Criteria Recommendation 1 values and risk values for *E. coli* in fresh water.

Align your compartment bag so compartment #1 is on the left and compartment #5 is on the right. Match the color sequence of your five compartments to one of these 32 rows:



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	2	3	4	5			
10mL	30mL	56mL	3mL	1mL			
0	0	0	0	0	0	28.70	Low Risk/Safe
0	0	0	1	0	10	51.40	Low Risk/ Probably Safe
0	0	0	0	1	10	47.40	
0	0	0	1	1	11	51.60	
0	1	0	0	0	12	56.40	
0	0	1	0	0	15	78.10	
0	0	0	1	1	20	63.20	
0	1	0	1	0	21	68.50	
0	0	1	1	0	21	66.40	
0	1	0	0	1	24	78.10	
0	0	1	0	1	24	81.20	
1	0	0	0	0	26	85.10	
0	1	0	0	1	32	83.80	
0	0	1	0	1	37	97.00	
0	0	0	1	0	31	113.60	
0	0	1	0	0	32	118.20	
1	0	0	0	1	34	125.30	
0	1	0	0	1	39	104.30	
0	0	1	0	0	40	109.40	
0	1	0	1	0	47	227.50	
0	0	1	0	1	52	147.30	
0	1	0	0	1	54	129.30	
0	0	1	1	0	56	171.40	
0	1	0	0	1	58	168.70	
0	0	1	0	1	84	211.90	
1	0	0	1	0	91	370.40	
0	1	0	0	1	96	376.80	
0	0	1	1	0	136	830.60	
1	0	0	0	1	171	563.50	
0	1	0	1	0	326	1455.50	
0	0	1	0	1	483	3519.10	
0	0	0	0	1	>1000	94351.00	
							Very Unsafe

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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 is also positive for Total Coliforms