



Aquagenx® CBT EC+TC (Compartment Bag Test) Most Probable Number (MPN) Kit

Instructions for Use: 1:10 Dilution for Surface and Recreational Waters

Overview

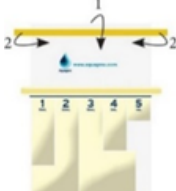
The Aquagenx CBT EC+TC MPN Kit simultaneously detects *E. coli* (EC) and Total Coliform (TC) bacteria in a 100 mL sample. When *E. coli* metabolize the substrate in Aquagenx's growth medium, the color of the water turns blue/blue green, indicating the presence of *E. coli*. If Total Coliforms are present, they fluoresce blue under UV light (365 nm). 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* or fecal bacteria.

Product documents: <https://www.aquagenx.com/cbt-ectc-product-documents/>

Shelf Life and Storage of Growth Medium

Aquagenx CBT EC+TC powder growth medium is stable up to three years after date of manufacture at 25° Celsius. Expiration date and lot number are printed on the medium packet. Store at 4-25° Celsius in a dry environment. Growth medium can be stored in a refrigerator. Cold chain for Aquagenx EC+TC growth medium is not required.

Summary of Test Procedures for 1:10 Dilution and Color Change Table

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

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

Optional UV Flashlight: Our growth medium is designed and tested to deliver reliable, accurate results for *E. coli* detection in 100mL water samples. For users who have positive test results in ambient light for *E. coli* and who would like additional verification, we offer this optional confirmation procedure: shine a UV light (365nm) on the sample. If it does fluoresce blue, this increases the likelihood the sample is positive for *E. coli*. This step is not required and does not indicate any concern with the primary test results. It simply provides additional corroboration for those seeking extra assurance.

Why a Yellow/Yellow Brown Compartment in Ambient Light Might be Positive for Total Coliforms Under UV Light:

Total Coliforms are a large group of different types of bacteria, many of which are not of fecal origin. Fecal bacteria and pathogens in drinking water are what can potentially make people and animals sick. *E. coli* is both a fecal bacteria and Total Coliform, but not all Total Coliforms are *E. coli*. Total Coliforms only give a general indication of the sanitary condition of a water supply. *E. coli* is the most specific indicator of fecal bacteria and pathogens in water, which is why the World Health Organization recommends testing for *E. coli* in its Drinking Water Quality Guidelines.

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

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

- Wear disposable plastic 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 10 mL water sample and pour into Thio-Bag. Record sample details such as date, time and location.

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*).

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

- Testing procedure should begin 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. It is most important to fill the two smallest compartments, compartments 4 and 5, up to the fill line.

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-shaped part of the clip across the width of bag so that the bottom edge of the clip is along the black fill line.
- Place rod-shaped part of the clip on the opposite side of Compartment Bag and snap into U-shaped part of the clip to lock in place.

7. 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 temperature between 25° - 37°C. 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.
- Note: over 40°C, some Total Coliforms will be inhibited, and the results might 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 *E. coli* and Total Coliforms.
- To detect thermotolerant (fecal) coliforms instead of Total Coliforms, incubate at 44.5°C (between 44-45°C) throughout an incubation period of 20-24 hours. Strict temperature control is required for this procedure.

Incubation Periods at Ambient Temperature Conditions:

35-37°C: 20 hours

31-34°C: 24-30 hours

25-30°C: 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 at a minimum of 25°C for up to 48 hours, depending on the temperature.

See "Incubation Period Guidance": <https://www.aquagenx.com/cbt-ectc-product-documents/>

8. Score MPN test results

- Hold the Compartment Bag next to the color-coded Aquagenx MPN Table on page 4 to score test results.
- ***E. coli* – view in ambient light:**
 - Yellow/yellow brown is negative for *E. coli* (absence)
 - Blue/blue green is positive for *E. coli* (presence). Positive results include any trace of blue/blue green, such as one or more specks or streaks of blue/blue green or blue/blue green sediment at bottom of a compartment.
- **Total Coliforms- shine UV light (365 nm) on Compartment Bag in dark environment:**
 - Blue fluorescence is positive for Total Coliforms. This includes samples that are yellow/yellow brown in ambient light that fluoresce blue under UV light.
- 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. Refer to Color Change Table on page 1.



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	Teal	Yellow	10	51.40	Low Risk/ Probably Safe
3	Yellow	Yellow	Yellow	Yellow	Teal	10	47.40	
4	Teal	Yellow	Yellow	Yellow	Yellow	11	51.60	
5	Yellow	Teal	Yellow	Yellow	Yellow	12	56.40	
6	Yellow	Yellow	Teal	Yellow	Yellow	15	78.10	
7	Yellow	Yellow	Yellow	Teal	Teal	20	63.20	
8	Teal	Yellow	Yellow	Teal	Yellow	21	68.50	
9	Teal	Yellow	Yellow	Yellow	Teal	21	66.40	
10	Yellow	Teal	Yellow	Teal	Yellow	24	78.10	
11	Yellow	Teal	Yellow	Yellow	Teal	24	81.20	
12	Teal	Teal	Yellow	Yellow	Yellow	26	85.10	
13	Teal	Yellow	Yellow	Teal	Teal	32	83.80	
14	Yellow	Teal	Yellow	Teal	Teal	37	97.00	
15	Yellow	Yellow	Teal	Yellow	Teal	31	113.60	
16	Yellow	Yellow	Teal	Teal	Yellow	32	118.20	
17	Teal	Yellow	Teal	Yellow	Yellow	34	125.30	
18	Teal	Teal	Yellow	Yellow	Teal	39	104.30	
19	Teal	Teal	Yellow	Teal	Yellow	40	109.40	
20	Yellow	Teal	Teal	Yellow	Yellow	47	227.50	Low Risk/Possibly Safe
21	Yellow	Yellow	Teal	Teal	Teal	52	147.30	
22	Teal	Teal	Yellow	Teal	Teal	54	129.30	
23	Teal	Yellow	Teal	Yellow	Teal	56	171.40	
24	Teal	Yellow	Yellow	Teal	Yellow	58	168.70	Intermediate Risk/Possibly Safe
25	Teal	Yellow	Teal	Teal	Teal	84	211.90	
26	Yellow	Teal	Teal	Yellow	Teal	91	370.40	High Risk/Probably Unsafe
27	Yellow	Teal	Yellow	Teal	Yellow	96	376.80	
28	Teal	Teal	Teal	Yellow	Yellow	136	830.60	Very High Risk/Unsafe
29	Yellow	Teal	Teal	Teal	Teal	171	563.50	
30	Teal	Teal	Teal	Yellow	Teal	326	1455.50	Very Unsafe
31	Teal	Teal	Teal	Teal	Yellow	483	3519.10	
32	Teal	Teal	Teal	Teal	Teal	>1000	94351.00	Very Unsafe

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