Aquagenx® CBT EC+TC (Compartment Bag Test)
Most Probable Number (MPN) Kit
Instructions for Use: Drinking Water

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 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*.

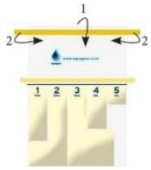
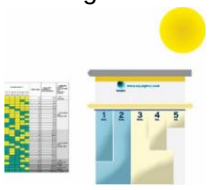
Instructions for testing surface and waste waters: https://www.aquagenx.com/dilutions-cbt-ectc/

Product documents: https://www.aquagenx.com/product-documents/

Shelf Life of Growth Medium
Aquagenx 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.

Storage of Growth Medium
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 CBT EC+TC MPN Kit

Collect 100 mL sample 	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 

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
E. coli	Negative	Negative	Positive	Positive
Total Coliforms	Negative	Positive	Positive	Positive

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Basis of Aquagenx CBT Most Probable Number (MPN) Table
The Aquagenx CBT MPN Table (page 4) 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 Compartment Bag.
See "Basis of Aquagenx MPN Table": https://www.aquagenx.com/product-documentation/

World Health Organization Guidelines for Drinking Water Quality, Table 5.4, Fourth Edition, 2017

E.coli classification (as decimal concentration/100)	Sanitary inspection risk score (susceptibility of supply to contamination from human and animal faeces) 0–2	3–5	6–8	9–10
< 1				
1–10				
11–100				
> 100				

Low risk: no action required	Intermediate risk: low action priority	High risk: higher action priority	Very high risk: urgent action required

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 100 mL water sample with 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 Thio-Bag to 100 mL fill mark. Record sample details such as date, time and location.

3. 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.

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 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.

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 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.

6. 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 temperature between 25°- 37°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/

7. 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.

8. 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.

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Aquagenx® CBT Most Probable Number (MPN) Table

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 1 10mL	2 30mL	3 56mL	4 3mL	5 1mL	MPN/100mL	Upper 95% Confidence Level/100mL	WHO Health Risk Category Based on MPN and Upper 95% Confidence Level
1						0.0	2.87	Low Risk/Safe
2						1.0	5.14	Intermediate Risk/ Probably Safe
3						1.0	4.74	
4						1.1	5.16	
5						1.2	5.64	
6						1.5	7.81	
7						2.0	6.32	
8						2.1	6.85	
9						2.1	6.64	
10						2.4	7.81	
11						2.4	8.12	
12						2.6	8.51	
13						3.2	8.38	
14						3.7	9.70	
15						3.1	11.36	Intermediate Risk/ Possibly Safe
16						3.2	11.82	
17						3.4	12.53	
18						3.9	10.43	
19						4.0	10.94	
20						4.7	22.75	
21						5.2	14.73	
22						5.4	12.93	
23						5.6	17.14	
24						5.8	16.87	
25						8.4	21.19	
26						9.1	37.04	
27						9.6	37.68	
28						13.6	83.06	High Risk/Possibly Unsafe
29						17.1	56.35	
30						32.6	145.55	High Risk/Probably Unsafe
31						48.3	351.91	
32						>100	9435.10	Unsafe
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