



Aquagenx® CBT AMR EC Kit
Antimicrobial Resistant (AMR)
ESBL *E. coli* Bacteria
Most Probable Number (MPN) Kit
Instructions for Use: Drinking & Surface Water

Overview

The Aquagenx CBT AMR EC MPN Kit detects and quantifies extended spectrum beta-lactam (ESBL) *E. coli* (EC) bacteria in a 100 mL sample. It uses a proprietary powder growth medium with a glucose substrate and the antibiotic Cefotaxime to detect antibiotic resistant strains of *E. coli*, specifically those with ESBL resistance. When *E. coli* metabolize the substrate in Aquagenx's growth medium, the color of the water in compartments turns blue/blue-green, indicating the presence of Cefotaxime resistant *E. coli*. MPN test results are obtained by easy color match using the Aquagenx color-coded MPN Table on page 4.

This test can be used for wastewater testing using a series of dilutions. See **Product Documents:**
<https://www.aquagenx.com/product-documents/>




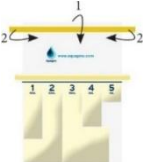
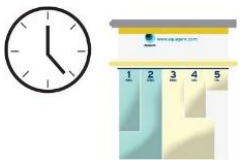


Shelf Life of Growth Medium

Aquagenx ESBL EC powder growth medium is stable up to one year 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 ESBL EC growth medium is not required.

Summary of Test Procedures for CBT EC+TC MPN Kit

<p>Collect 100 mL sample</p> 	<p>Add powder growth medium</p> 	<p>Pour sample into compartment bag</p> 	<p>Roll down Whirl-Pak seal and attach plastic clip seal</p> 
<p>Incubate 20-48 hours</p> 	<p>View and score EC test results in ambient light</p> 	<p>Decontaminate sample</p> 	

How to Interpret Color-Change Test Results

<p>Color of compartment in Compartment Bag</p>	<p>Yellow/Yellow Brown in ambient light</p>	<p>Blue/Blue Green in ambient light</p>
<p>ESBL <i>E. coli</i></p>	<p>Negative</p>	<p>Positive</p>

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.

See "Basis of Aquagenx MPN Table": <https://www.aquagenx.com/product-documentation/>

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 ESBL EC 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 to close Thio-Bag .
- 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 other container during the incubation period.
- Ambient temperature incubation works at any temperature between 25°- 37°C for detection of ESBL *E. coli*
- 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.
- For regulatory compliance purposes, samples must be incubated at 35-37°C for 20-24 hours to detect and quantify *E. coli*.

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.

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.
- **ESBL *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.
- 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*.
- 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.

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):



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

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