



Compartment Bag Test (CBT) for *E. coli* Instructions for Use: Drinking Water

CBT *E. coli* Kit Components



100 mL sample bottle in CBT *E. coli* Kit 10-Pack



100 mL Thio Bag in CBT *E. coli* Kit 50-Pack



E. coli growth medium



Compartment bag



Seal Clip



Chlorine tablets in CBT *E. coli* Kit 10-Pack

Additional Equipment Needed: Disposable plastic gloves; disinfectant cleaning solution, paper towels or wipes to clean work area; CBT *E. coli* Kit 50-Pack users source their own liquid bleach or chlorine to disinfect sample after scoring for safe disposal.

Compartment Bag Volumes and *E. coli* Growth Medium



The compartment bag has five compartments of different volumes that total 100 mL:

- 1 = 10 mL
- 2 = 30 mL
- 3 = 56 mL
- 4 = 3 mL
- 5 = 1 mL











E. coli growth medium contains a chromogenic substrate: 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid (X-Gluc)

Shelf Life and Storage of *E. coli* Growth Medium

- *E. coli* growth medium (test bud) is stable up to 2-years after date of manufacture at 25-30° Celsius and provides positive results over this time period.
- Recommended storage temperature for *E. coli* growth medium is 15-25° Celsius. It is safe to store the test buds in a refrigerator.
- Protect *E. coli* growth medium from bright light.

How to Use CBT – Quick Summary

STEP 1: Collect 100 mL water sample with bottle or Thio Bag		STEP 2: Add <i>E. coli</i> growth medium to bottle or Thio Bag		STEP 3: Slowly pour sample into compartment bag for incubation period		STEP 4: Score test results using MPN Table on page 4
 	<p>Testing should begin within six hours of sample collection.</p> <p>Sample can be stored for up to four days below 10° Celsius but not frozen.</p>	 <p>Do not add test bud to sample until sample is ready to pour into compartment bag.</p>	<p>Dissolve <i>E. coli</i> growth medium in sample for 10-12 minutes. Only the medium dissolves, not its plastic carrier. Then, pour sample into the compartment bag without further delay.</p>	 <p>While pouring, gently squeeze bag from its sides and tilt to move liquid between compartments and to get liquid levels to the top of the fill line.</p>	<p>Incubation period is 24-48 hours depending on ambient temperature. See incubation period recommendations on page 3.</p>	 <p>Compartments are considered positive for <i>E. coli</i> with any trace of blue or blue green, even if just a speck of color or as precipitate at the bottom of a compartment.</p>

1. Prepare work area

- Sanitize your work area with a disinfectant cleaning solution and disposable paper towels or wipes

2. Collect 100 mL water sample

- Plastic gloves, thin and disposable, are recommended. Avoid touching inside of bottle, lid or Thio Bag with bare hands.
- Fill sample bottle or Thio Bag to 100 mL fill mark, record sample details

3. Add *E. coli* growth medium to sample

- Open growth medium pouch and add test bud to sample. Leave white desiccant in foil pouch.
- Do not touch growth medium with bare fingers or hands
- Dissolve medium in sample for 10-12 minutes by periodically swirling the bottle or squeezing the test bud in the Thio Bag to promote release of the medium from its plastic carrier.
- The medium dissolves from its plastic carrier. When medium is completely dissolved, the plastic carrier turns white or nearly white.

4. Pour sample into compartment bag

- Label bag before filling according to your sample identification requirements
- Tear off perforated seam at top of bag
- Rub sides and top of bag together in each compartment to open them so water easily runs into compartments
- Use white tabs at top of bag to hold it open while pouring
- Slowly pour sample into bag while gently tilting and squeezing bag to distribute sample amongst five compartments
- Fill evenly to the top of the fill line. Leave test bud carrier in bottle or Thio Bag while pouring.

TIP: The white particles in sample bottle and white tablet in Thio Bag are sodium thiosulfate, which neutralizes residual chlorine in sample. Do not remove.

Dissolved medium indicated by its carrier turning white.



TIP: Rub sides and top of compartment bag together to open each compartment before pouring in sample. Recommend two people handle pouring sample.

TIP: CBT Kits include one reusable seal clip. Aquagenx sells extra seal clips separately.

5. Seal compartment bag

- Attach seal clip across the bag above the fill line and below the compartment top openings. Do this by placing the U-shape across width of the bag above liquid level along the fill line but below compartment openings. Snap rod-shaped part of the clip from other side of bag into U-shape to lock in place across the bag. Close the top of the bag with the yellow Whirl-Pak seal and then roll down the bag toward the seal clip. After attaching clip, bag can be kept vertical or horizontal.

6. Incubation Period

- The CBT works at variable temperatures. Constant temperature control in an incubator is not required, but recommended in cooler temps if available.
- Ambient temperature incubation period works at 25°C and up to 44.5°C.
- Below 25°C, use a portable incubator or find a warm location at or above 25°C for incubation.

TIP: During the incubation period, CBTs develop an odor. We recommend placing CBTs in another sealed plastic bag or container during the incubation period.

Incubation Period Time and Temperature Recommendations:

35-44.5°C: Incubate 20-24 hours
 31-34°C: Incubate 24-30 hours
 25-30°C: Incubate 40-48 hours

Below 25°C: Incubate in a portable incubator, preferably at 35-37°C for 48 hours or put in or near another heat source. In addition to portable incubators, samples can be maintained at 25°C to 44.5°C using: thermal coolers/bags; proximity to heat sources for humans and food; poultry or reptile incubator, or near refrigerator coils or room heating vents.

7. Score and record test results



Yellow/Yellow-Brown = Absence of *E. Coli*
Blue/Blue-Green = Presence of *E. coli*

- Align compartments in correct sequence to MPN Table on page 4 to determine *E. coli* concentration. Compartment #1 is on your left.
- Concentration of *E. coli* in sample is estimated from the combination of positive and negative compartments in bag, providing Most Probable Number (MPN) estimate of *E. coli* per 100 mL
- Hold bag up to read results in each compartment
- Yellow/yellow-brown indicates negative (absence) for *E. coli*
- Blue/blue-green indicates positive (presence) for *E. coli*. Any trace of blue or blue/green in a compartment is considered positive, even just specks of color or just sediment in bottom of compartment.
- Match color sequence of five compartments to one of 32 rows in MPN Table. Record MPN result.

8. Decontaminate sample

- CBT Kit 10-Pack users add three chlorine tablets included in the kit to top of compartment bag. Seal bag with seal clip. Agitate sealed bag until chlorine dissolves. CBT Kit 50-Pack users source and add 1-2 mL of liquid bleach (NaOCl) or sufficient free chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) to provide about 100 milligrams of free chlorine.
- After 45 minutes, pour contents into a sink, toilet or hole in ground and safely dispose the empty compartment bag
- Retain seal clip for reuse

WHO Guidelines for Drinking Water Quality, 4th Edition, 2011

Health Risk Category	<i>E. coli</i> CFU per 100 mL
Safe	<1
Intermediate Risk/Probably Safe	1-10
High Risk/Probably Unsafe	>10-100
Very High Risk/Unsafe	>100

Most Probable Number (MPN) Table

The MPN Table is based on World Health Organization "Guidelines for Drinking Water Quality," 4th Edition. Table 5.4 in the Guidelines has risk categories of drinking water based on *E. coli* levels as ranges: 0/100 mL = Safe; 1-10/100 mL = Intermediate Risk; 11-100/100 mL = High Risk; and >100/100 mL = Very High Risk. The general consensus is drinking water should contain no *E. coli*, but in some countries *E. coli* numbers of up to 10 or 20/100 mL may be tolerated as being of intermediate but allowable risk.

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 #					MPN/100mL	Upper 95% Confidence Interval/100mL	Health Risk Category Based on MPN and Confidence Interval
1	2	3	4	5			
10mL	30mL	56mL	3mL	1mL			
					0.0	2.87	Low Risk/Safe
					1.0	5.14	Intermediate Risk/ Probably Safe
					1.0	4.74	
					1.1	5.16	
					1.2	5.64	
					1.5	7.81	
					2.0	6.32	
					2.1	6.85	
					2.1	6.64	
					2.4	7.81	
					2.4	8.12	
					2.6	8.51	
					3.2	8.38	
					3.7	9.70	
					3.1	11.36	
					3.2	11.82	
					3.4	12.53	
					3.9	10.43	
					4.0	10.94	
					4.7	22.75	
					5.2	14.73	
					5.4	12.93	
					5.6	17.14	
					5.8	16.87	
					8.4	21.19	
					9.1	37.04	
					9.6	37.68	
					13.6	83.06	
					17.1	56.35	
					32.6	145.55	
					48.3	351.91	
					>100	9435.10	
							High Risk/Possibly Unsafe
							High Risk/Probably Unsafe
							Unsafe