



Aquagenx

Safe water for anyone, anywhere, anytime

Compartment Bag Test (CBT) Instructions for Use: Drinking Water

A video on how to use the CBT is on our website: <http://www.aquagenx.com/how-to-use-the-cbt/>

CBT Kit Components



100 mL sample bottle in CBT I Kit



100 mL Thio Bag in CBT II Kit



E. coli growth medium



Compartment bag



Seal clip



Chlorine tablets in CBT I Kit

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

- Shelf life of *E. coli* growth medium (test bud) is one year from date of manufacture
- Overall, it is best to store the *E. coli* test buds at temperatures of 25°C or less. Test buds can be kept in refrigerators but not frozen. Extended periods of storage time for months at temperatures in the 30s and 40s Celsius may result in some decrease in performance but will still support the growth of *E. coli* bacteria and produce the typical color change to blue or blue-green, perhaps with lower efficiency. Test buds stored in warmer temperatures for extended periods can become sticky and more difficult to handle.

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° C but not frozen.</p>		<p>Dissolve <i>E. coli</i> growth medium for 15 minutes. Only the medium dissolves, not its plastic carrier. Add sample with dissolved medium to compartment bag within 30 minutes.</p>	 <p>Gently squeeze bag from its sides and tilt to move liquid between compartments to get liquid levels to fill line.</p>	<p>Incubation period is 24-48 hours depending on ambient temperature. See incubation period recommendations on page 3.</p>	 <p>Positive compartments are blue or blue green in the liquid or as a precipitate at the bottom of compartment.</p>	

1. Prepare work area

- Sanitize your work area with a disinfectant cleaning solution

2. Collect 100 mL water sample

- Plastic gloves 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
- Do not touch growth medium with bare fingers or hands
- Dissolve medium in sample for about 15 minutes by periodically swirling the bottle or squeezing the medium bud in the sample 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 sampling requirements
- Tear off perforated seam at top of bag
- Rub two sides 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 across bag to fill line. Leave test bud in bottle or Thio Bag while pouring.

5. Seal bag

- Attach seal clip across the bag, above the liquid levels 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. After attaching clip, bag can be kept vertical or horizontal. Close the top of the bag with the yellow Whirl-Pak seal and then roll down the bag toward the external clip.

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 of compartment bag together to open each compartment before pouring in sample. Recommend two people handle pouring sample.

TIP: CBT Kits include one seal clip. You can seal 2-3 bags together at the same time and reuse clips. Aquagenx also sells extra seal clips.

6. Incubation Period

- The CBT works at variable temperatures. Constant temperature control in an incubator is not required but recommended if available.
- Ambient temperature incubation period works at 25°C and up to 44.5 °C.
- Between 25-30°C, extend incubation period to 48 hours. Alternatively, put sample in insulated container, or keep sample near any heat source to maintain the temperature anywhere between 25°C to 44.5°C.
- In colder temperatures, use portable incubators, such as poultry or reptile egg incubators

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

7. Score and record test results

- Align compartments in correct sequence to MPN Table on page 4 to determine *E. coli* concentration.
- 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
- 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, in liquid or as sediment in bottom of compartment.
- Record MPN result



Yellow/Yellow-Brown = Absence of *E. Coli*

Blue/Blue-Green = Presence of *E. coli*

8. Decontaminate

- Open bag and add chlorine tablets included in CBT I Kits to top of bag. Seal bag with two-piece clip near bag top. Agitate sealed bag until chlorine dissolves. Let stand for 45 minutes. CBT II Kits users can source their own liquid bleach or chlorine tablets to decontaminate sample.
- 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

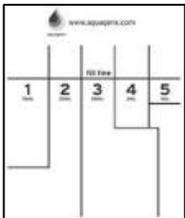
Health Risk Category	<i>E. coli</i> CFU* per 100 mL
Safe	<1
Intermediate Risk/Probably Safe	1-10
High Risk/Probably Unsafe	>10-100
Very High Risk/Unsafe	>100

*MPN and CFU (colony forming units) are equivalent terms, but MPN is obtained in quantal tests such as the CBT and CFU is obtained in colony-based tests such as membrane filtration.

Most Probable Number Table

The MPN Table represents 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	High Risk/Possibly Unsafe
					17.1	56.35	High Risk/Probably Unsafe
					32.6	145.55	
					48.3	351.91	Unsafe
					>100	9435.10	Unsafe