

Compartment Bag Test (CBT) Instruction Packet for Surface Water Testing of *E. coli*

The Compartment Bag Test (CBT) is a quick and semi-quantitative method to measure the indicator bacteria, *Escherichia coli* (*E. coli*). Just about anyone can use the CBT Kit with just a little training. The Aquagenx CBT quantifies the Most Probable Number (MPN) of *E. coli* bacteria in a 100 mL water sample to screen surface water to determine potential at-risk areas.

The basic steps of the test are as follows:



Collect Sample → Mix Medium → Pour into Bag → Incubate Sample → Score Results

A video on how to use the CBT can be found at: <http://www.aquagenx.com/how-to-use-the-cbt/>

Everything you need to measure *E. coli* levels in an ambient water sample is in the enclosed testing kit. Verify that you have everything you need in your kit below before testing:



(a) 150 mL Sterile Bottle (b) Compartment Bag (c) *E. coli* Growth Media (d) 90 mL Sterile Water



(e) Reusable Bag Clip (f) Disposable Gloves (L) (g) Outer Bag (h) Plastic Syringe

Please follow manufacturers recommendations for safe use and disposal. This test should be administered by adults or under close adult supervision after careful evaluation of the test procedure and proper training.

Refer to the Aquagenx website for additional safety information, including the CBT Material Safety Data Sheet (<https://www.aquagenx.com/wp-content/uploads/2016/07/MSDS-Aquagenx-CBT-I-Kit.pdf>). For any other questions regarding product safety, please call the manufacturer, Aquagenx, directly at 919-590-0343.

Compartment Bag Test (CBT) Instructions

STEP 1: COLLECT WATER SAMPLE

Collect approx. 100 mL water sample in 150 mL sterile plastic sample bottle (a). Leave some headspace in bottle to allow for mixing. Do Not Stick your fingers in bottle when opening. Scoop water sample and cap just below the surface of the water or if using with sample pole, cap immediately after collection of sample. If the sample cannot be analyzed immediately, place sample on ice and begin test within 8 hours of collection. Use Data Sheet (Attachment 2) to record all information for the test including sampling and testing.

TIP: Disposable plastic gloves (one pair included in test kit) are highly recommended for sampling and handling of the test kit and your water samples.

STEP 2: MAKE 10% SAMPLE DILUTION

Sanitize your work area with a bleach spray (i.e. Chlorox). Homogenize sample bottle (a) by gently shaking approximately 24 times. Open sample bag carefully, set on stable surface and subsample 10 mLs using the plastic syringe (h) by pulling the plunger on syringe up while tip is in contact with sample in the bottle. Take care not to touch the inside of the sample bag with your fingers to prevent sample contamination. Place 10 mL sample from plastic syringe (h) into the 90 mL bottle of sterile deionized water (d). THIS IS YOUR 10% TEST SAMPLE. The sample is diluted so a higher concentration of *E. coli* can be measured if needed.

STEP 3: ADD *E. COLI* MEDIA PELLET TO SAMPLE

Add *E. coli* growth media (c) to your 10% test sample bottle (d) that you prepared in STEP 2 above. Do not touch the *E. coli* growth medium pellet (c) with your fingers. Dissolve the *E. coli* growth medium for 15 minutes by periodically swirling the bottle with the *E. coli* growth media (c). Only the medium dissolves, not the plastic pellet. When medium is completely dissolved, the *E. coli* growth media (c) turns white or nearly white. Please allow 15 minutes for media pellet to mix with sample, but do not wait more than 30 minutes before progressing onto STEP 4.

STEP 4: POUR SAMPLE INTO COMPARTMENT BAG

Shake 10% test sample bottle (d) with *E. coli* growth media (c) a minimum of 24 times prior to adding to the compartment bag (b). Slowly pour water sample into compartment bag (b) while gently tilting and squeezing bag to distribute sample in the 5 compartments. Do not transfer the *E. coli* growth media (c) into the bag.

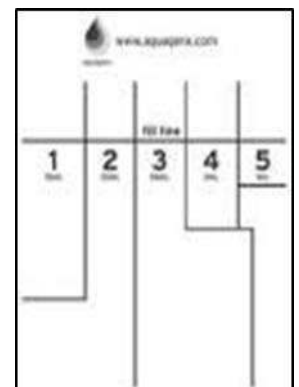
TIP: Rub sides of compartment bag together to open each compartment before pouring in sample. Recommend two people handle pouring sample.

It is **essential** that the sample is distributed up to the fill line of each compartment. The bag has 5 compartments with different volumes of sample. It is this design that allows for estimation of *E. coli* concentrations.

STEP 5: SEAL THE BAG

The bag clip (e) has 2 pieces – a rod and outer sheath. To seal the bag after the 100 mL sample is added, take the rod and place on one side of the bag and on the direct opposite side of the bag, connect the sheath to the rod, trapping the sample in the bag.

IMPORTANT: Make sure the bag clip (e) is attached at, or just below the fill line to prevent sample from overflowing to adjoining compartments. Refer to "Fill Line" on photo at right.



STEP 6: INCUBATE THE COMPARTMENT BAG FOR 24-48 HOURS

Place sealed compartment bag with sample and clip into outer leak proof bag (g). During incubation, if *E. coli* are present, an odor from this process may be unpleasant. The outer leak proof bag (g) will prevent odors from escaping during incubation. The compartment bag (b) must be incubated for 24 – 48 hours depending on the incubation temperature.

- The CBT works at variable temperatures between 25-44.5 °C.
- Constant temperature control in an incubator is not required but can be used if available with a suggested incubation of 35.0 to 44.5 °C for 24 hours.
- Without access to an incubator, the sample can be placed in an insulated container, or kept near any heat source or area where temperature is at least 25 °C (78 °F).
- For incubation at the lower temperature range of 25-30 °C, extend the incubation period to 48 hours.
- If you do not have a thermometer you can use weather forecasts to determine temperature. It is recommended that the low temperature be used to determine incubation time.

Detailed Incubation Temperatures:

35-44.5 °C or >93 °F	Incubate 20-24 Hours
31-34 °C or 88-93 °F	Incubate 24-30 Hours
25-30 °C or 78-87 °F	Incubate 40-48 Hours

STEP 7: READ THE RESULTS

The concentration of *E. coli* in the test sample is estimated from the combination of positive and negative compartments in the compartment bag (b), providing the Most Probable Number (or MPN) of *E. coli* per 100 mL. Refer to the MPN Table in **Attachment 1** to determine *E. coli* concentration.

- Align the compartment bag (b), with Compartment #1 on the left and Compartment #5 on the right.
- Hold bag up to read results (you can keep it in the outer bag to read if necessary).
- Yellow/yellow-brown indicates negative (absence) for *E. coli*.
- Blue/blue-green indicates positive (presence) for *E. coli*. Any trace of blue should be interpreted as positive.
- Use the data sheet in **Attachment 2** to record your test information and results. Use a separate sheet for each sample tested.



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

STEP 8: DISPOSAL

Open compartment bag (b) and add approximately 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.

Let stand for 45 minutes. After 45 minutes, pour contents into a sink, toilet or adhere to local municipal codes. The empty bag after chlorine addition is safe to dispose in municipal trash.

STEP 9: INTERPRET THE RESULTS

Category/Level	<i>E. coli</i> MPN* per 100 mL
None to Low	<126
Moderate	>126 but <410
High to Very High	>410

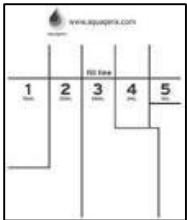
*MPN = Most Probable Number. MPN is a statistical endpoint based on probability of the combination of Positive compartments and Volume of sample in each compartment. Although not exactly equivalent, it is interchanged with the endpoint Colony Forming Units (CFU) per 100 mL that is used to express results for a membrane filter and colony count for bacteria. This table is loosely based on the recommended Statistical Threshold Value (STV) from the 2012 Recreation Water Quality Criteria for Bacteria and *E. coli* for surface water samples used for contact recreation.

ATTACHMENT 1: MOST PROBABLE NUMBER (MPN) TABLE

The MPN Table is adapted for measuring *E. coli* bacteria in surface waters. The CBT Kit's upper detection limit is 483 *E. coli* /100 mL when analyzing a 10 mL sample. The MPN Table is adjusted to account for a 10% sample dilution.

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.

EXAMPLE: If Compartment #1 is Blue, Compartment #2 is Blue, Compartment #3 is Yellow, Compartment #4 is Yellow, and Compartment #5 is Blue, the MPN is 39 MPN/100 mL.



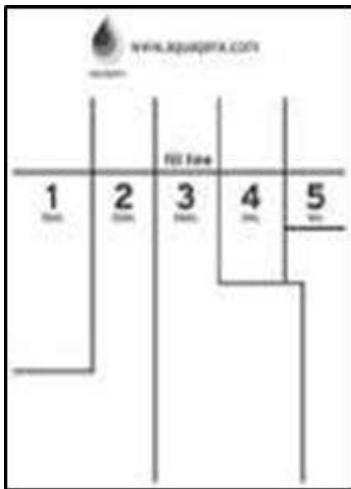
Compartment#					MPN/100mL	Upper 95% Confidence Limit/100mL	Comments/Observations:
1 10mL	2 30mL	3 56mL	4 3mL	5 1mL			
Yellow	Yellow	Yellow	Yellow	Yellow	0	28.70	
Yellow	Yellow	Yellow	Blue	Yellow	10	47.40	
Yellow	Yellow	Yellow	Yellow	Blue	10	51.40	
Blue	Yellow	Yellow	Yellow	Yellow	11	51.60	
Yellow	Blue	Yellow	Yellow	Yellow	12	56.40	
Yellow	Yellow	Blue	Yellow	Yellow	15	63.20	
Yellow	Yellow	Yellow	Blue	Blue	20	66.40	
Blue	Yellow	Yellow	Blue	Yellow	21	68.50	
Blue	Yellow	Yellow	Yellow	Blue	21	78.10	
Yellow	Blue	Yellow	Blue	Yellow	24	78.10	
Yellow	Yellow	Yellow	Yellow	Blue	24	81.20	
Blue	Yellow	Yellow	Yellow	Yellow	26	83.80	
Blue	Yellow	Yellow	Blue	Yellow	32	85.10	
Yellow	Blue	Yellow	Yellow	Blue	37	97.00	
Yellow	Yellow	Blue	Yellow	Yellow	31	104.30	
Yellow	Yellow	Yellow	Blue	Yellow	32	109.40	
Blue	Yellow	Yellow	Blue	Yellow	34	113.60	
Blue	Blue	Yellow	Yellow	Yellow	39	118.20	
Blue	Yellow	Yellow	Blue	Yellow	40	125.30	
Yellow	Blue	Blue	Yellow	Yellow	47	129.30	
Yellow	Yellow	Yellow	Blue	Blue	52	147.30	
Blue	Yellow	Yellow	Blue	Yellow	54	168.70	
Blue	Yellow	Blue	Yellow	Yellow	56	171.40	
Blue	Yellow	Yellow	Blue	Blue	59	211.90	
Blue	Blue	Yellow	Blue	Blue	85	227.50	
Yellow	Blue	Blue	Yellow	Blue	91	370.40	
Yellow	Yellow	Blue	Blue	Yellow	96	376.80	
Blue	Yellow	Yellow	Yellow	Yellow	136	563.50	
Yellow	Yellow	Blue	Blue	Blue	171	830.60	
Blue	Blue	Blue	Yellow	Yellow	326	1455.50	
Blue	Blue	Blue	Blue	Yellow	483	3519.10	
Blue	Blue	Blue	Blue	Blue	>1000	94351.00	

ATTACHMENT 2: PUERTO RICO WATER QUALTY MONITORING DAY, *E. Coli* SAMPLE DATA SHEET

Name		Organization	
Phone Number		Email	
Name of Waterbody		Location of Sampling Station	
Sampling Date		Sampling Time	
CBT Test Start Date		CBT Test Start Time	
Air Temp at Start of Test		Was 10% Sample Tested?	
CBT Test End Date		CBT Test End Time	
Air Temp at End of Test		Sample Bag Properly Disposed?	

RESULTS:

Make sure Compartment Bag is oriented to match the picture below when interpreting results:



A positive result is blue/blue-green
A negative result is yellow/yellow-brown



1	2	3	4	5
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Enter a "+" or "-" in the 5 boxes above.

Find the same pattern on the MPN Table and record result in the box below:

RESULT, *E. Coli* MPN/100 mL =

Within 24 hours of the completed test, please provide a copy of this completed sheet, SJBEF via the following email:

diademonitoreo@estuario.org