



CBT *E. coli* Kit FAQ

Sample Storage

For 100 mL water samples that are collected, transported and stored before the *E. coli* medium is added, it is best if the sample is stored no more than four days and that storage temperatures are at or below 10° Celsius.

Samples can be transported and stored for time periods of up to 30 hours at ambient temperatures into the 30s Celsius without causing changes in the detectability and quantification of *E. coli*.

At higher ambient temperatures for extended periods of time greater than 30 hours, it is possible *E. coli* bacteria will die or become unculturable. Therefore, starting the test as soon as conveniently possible after the water sample has been collected is recommended. **We recommend testing begin within six hours of sample collection.**

When to Add *E. coli* Test Bud to Sample

The test bud should NOT be added to the sample until the sample is ready to go into the compartment bag. The time between adding the test bud to the sample and then pouring the sample into the compartment bag should be 10-12 minutes.

Incubation Period

Follow the CBT Instructions for Use. Temperatures are in Celsius:

Ambient Temperature	Incubation Period
35-44.5°C	20-24 hours
31-34°C	24-30 hours
25-30°C	40-48 hours
Below 25°C	Recommend portable incubator or other incubation method

When the temperature gets too low, roughly below 20° C, *E. coli* may not grow at these lower temperatures and they become inactive.

When temperatures are above 45° C for extended periods of time, there may be some decrease in *E. coli* quantification because such higher temperatures may be harmful to *E. coli* and inhibit their growth.



Incubating for 48 hours vs. 72 hours

CBTs can be incubated for 72 hours if temperatures remain cold, such as at or below 25° C. However, they must be at 25° C for at least 48 hours to give reliable results from the growth of the bacteria.

There is no adverse effect of incubating too long up to a point. However, incubating longer than necessary presents no advantage for final test results.

When Ambient Temperature Varies Between Day and Night

The CBT can be incubated at ambient temperatures between 25-44.5° C and provide reliable results in 24 to 48 hours. The longer incubation time of about 48 hours is needed when the temperature remains between 25° C and 30° C. This is because the bacteria are multiplying and feeding at a slower rate. Nevertheless, the bacteria will feed and multiply effectively even if the temperature is cycling from day to night anywhere in this temperature range, but with slower multiplication and feeding when temperatures remain in the low end of this range.

If temperatures drop below 25° C but then go back to or above 25° C, the bacteria will remain present, multiply and feed, but at slower rates. However, when the temperature gets too low, roughly below 20° C, they may not grow at all at these lower temperatures and they become inactive. If and when temperatures go back above 20° C, and even more so when they go above 25° C, they will feed and multiply again. Therefore, it is possible for the CBT to provide reliable results where temperatures cycle at a lower range that includes 25° C and higher, if they are incubated longer to give the bacteria more time to feed and grow when the temperature is at or above 25° C.

When Ambient Temperature Remains Below 25° Celsius

When temperatures remain below 25° C, it is necessary to place CBT samples in locations that provide elevated temperatures for the bacteria to feed and grow. Constant temperature control is not required, but samples need to be maintained anywhere between 25-44.5° C.

This can be done by using portable incubators. Aquagenx provides a portable incubator with three types of power sources. Details are on our website: <https://www.aquagenx.com>

Other incubator alternatives are putting samples in or near another heat source, reptile or poultry incubators, thermal bags and coolers, warm rooms.