



Aquagenx® CBT EC+TC Kit Incubation Period and Temperatures

Ambient Temperature Incubation

- Ambient temperature incubation works at any temperatures between 25°- 44.5°C for detection of *E. coli* and/or total coliforms.
- The CBT EC+TC Kit works at variable temperatures. Constant temperature control in an incubator is not required. However, at cooler temperatures, constant temperature incubation is recommended, if available.
- Note: over 40°C, some total coliforms will be inhibited, and the results may not be accurate for total coliform analysis.
- For regulatory compliance purposes, samples must be incubated at 35-37°C for 20-24 hours to detect *E. coli* and total coliforms.

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

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 20 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, some *E. coli* 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 ambient 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. If a portable incubator is unavailable, other incubation methods can be used to maintain CBT samples between 25-44.5°C:

- In or near a heat source such as a cook stove or other source of heat for cooking or keeping people warm
- Inside warm rooms at temperatures of 25°C or higher
- Inside thermal picnic coolers or insulated bags
- Under an incandescent light bulb in a box
- Warming or incubation-type units that keep food warm
- Reptile or poultry incubators
- Yogurt makers
- Exposing to sunlight as a source of heat, but covered with black cloth, black plastic or black metal that absorbs heat, warms the sample and prevents direct sunlight exposure of the bag.