Aquagenx® CBT EC+TC Presence/Absence (P/A) Kit Instructions for Use: Drinking Water

Overview

The Aquagenx CBT EC+TC P/A Kit simultaneously detects *E. coli* (EC) and Total Coliforms (TC) in a 100 mL sample. It uses a proprietary powder growth medium with a glucose substrate called X-Gluc. When *E. coli* metabolize this substrate in Aquagenx's growth medium, the color of the water turns blue, indicating the presence of *E. coli*. The growth medium also contains a fluorogenic galactoside substrate called MUGal. If total coliforms are present, they metabolize this fluorogenic substrate and the sample fluoresces blue under a UV light (365 nm). Presence/Absence (P/A) test results are obtained by easy-to-see color change. The total coliform group of bacteria includes *E. coli*, which is a fecal coliform as well as a thermotolerant coliform. Not all total coliforms are *E. coli*.

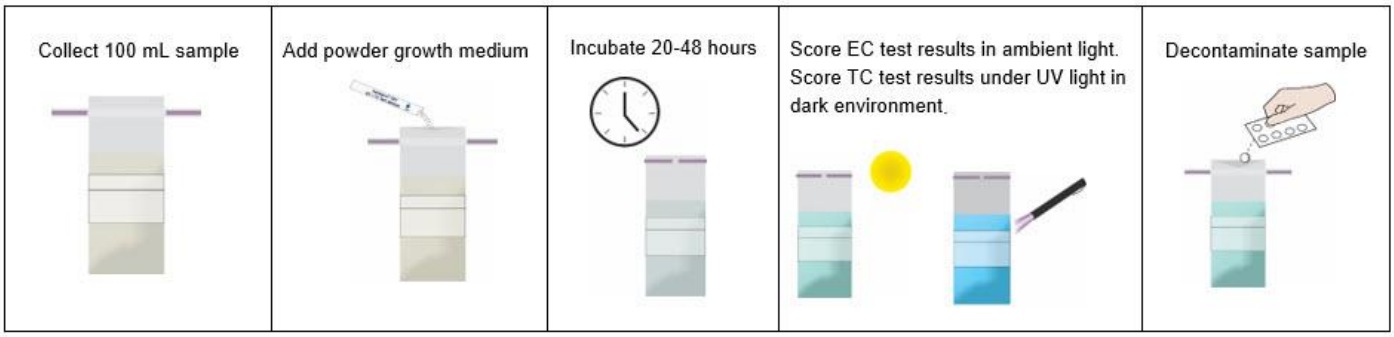
Shelf Life

Aquagenx EC+TC growth medium is stable up to two-years after date of manufacture at 25° Celsius. Expiration date and lot number are printed on back of medium packet.

Storage

Cold chain for Aquagenx EC+TC growth medium is not required. Recommended storage temperature for growth medium is 10-25° Celsius. Growth medium can be stored in a refrigerator.

Summary of Test Procedures for CBT EC+TC P/A Kit



How to Interpret Color-Change Test Results

Color in Thio-Bag	Yellow/Yellow Brown in ambient light and does not fluoresce blue under UV light	Yellow/Yellow Brown that … fluoresces blue under UV light	Blue/Blue Green in ambient light	Blue/Blue Green that… fluoresces blue under UV light
E. coli	Negative	Negative	Positive	Positive
Total Coliforms	Negative	Positive	Positive	Positive

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Procedural Notes

1. Sanitize work area with disinfectant cleaning solution, paper towels or wipes.

2. Collect 100 mL water sample with Whirl-Pak™ Thio-Bag™

- Label bag or attach barcode asset tag to Thio-Bag. The white tablet in Whirl-Pak Thio-Bag is sodium thiosulfate, which neutralizes residual chlorine in sample. Do not remove.
- Wearing disposable, thin plastic gloves is recommended. If you don't have gloves, avoid touching inside of Thio-Bag with bare hands.
- Fill Thio-Bag to 100 mL fill mark. Record sample details.

3. Add Aquagenx® EC+TC growth medium to sample in Whirl-Pak Thio-Bag

- Open growth medium packet with scissors and pour powder growth medium into Thio-Bag. Do not touch growth medium with bare fingers or hands. Growth medium should not be added to sample before you are ready to begin test procedure.
- Roll down Whirl-Pak seal and close Thio-Bag shut.
- Dissolve medium in sample. Gently swirl the bag until the medium is completely dissolved. You can squeeze any clumps of powder to help them dissolve more quickly.

4. Incubation Period and Temperatures

- During the incubation period, CBTs can develop an odor. To control odor, place CBTs in another sealed plastic bag or container during the incubation period.
- Ambient temperature incubation works at any temperatures between 25°- 44.5°C for detection of *E. coli* and/or total coliforms.
- Because the CBT 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.
- The CBT also can be used to detect thermotolerant (or fecal) coliforms instead of total coliforms, if the CBT samples are incubated at a temperature of 44.5°C (between 44-45°C) throughout an incubation period of 20-24 hours. Strict temperature control is required for this procedure.

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

Below 25°C: Incubate in a portable incubator at 35-37°C for 24 hours or put in or near another heat source for up to 48 hours, depending on the temperature.
Over 40°C: Some coliforms will be inhibited, and the results may not be accurate for total coliforms.

5. Score and record P/A test results

- ***E. coli:***
  - Yellow/yellow-brown indicates negative (absence) for *E. coli.*
  - Blue/blue-green indicates positive (presence) for *E. coli*. These include:
    - Any trace of blue/blue-green, or just specks of blue/blue-green, or just blue/blue-green sediment at bottom of Thio-Bag is considered positive.
- **Total Coliforms - shine UV light (365 nm) on Thio-Bag in a dark environment:**
  - Samples that fluoresce blue are positive for total coliforms. These include:
    - Any samples that are yellow/yellow brown in color that fluoresce blue under UV light.
    - Any samples that are blue/blue-green in color are positive for *E. coli* and by definition are positive for total coliforms.

6. Decontaminate sample

- Add 4 mL of liquid bleach (NaOCl) or sufficient chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) to Thio-Bag to provide about 200 milligrams of free chlorine.
- After 30 minutes, pour contents into a sink, toilet or hole in ground and safely dispose the empty Thio-Bag.

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