## Overview

The device tests for the presence or absence of E. Coli and Total Coliforms in water by incubating a water sample containing β-glucuronidase and β-glucuronidase nutrients. The device estimates the amount of E. Coli is present based on the incubation time taken until the sample changes to a colour confirming the presence of E. Coli.

## Concept

Colilert, Colilert 18, Colisure, Colitag and several other tests for E. Coli use two nutrient-indicators, ONPG and MUG which are metabolised by the coliform enzyme β-galactosidase and the E. coli enzyme β-glucuronidase, respectively. As coliforms grow in the E. Coli tests, they use β-galactosidase to metabolize ONPG and change the water colour from colourless to yellow. E. coli use β-glucuronidase to metabolize MUG and create fluorescence. Since most noncoliforms do not have these enzymes, they are unable to grow and interfere. These tests have been approved by the EPA for testing drinking water for many years.

The shade of yellow and fluorescence produced that indicates at least 1 CFU per 100mL is compared to a Comparator for the test.

The tests can take between 16 -24 hours to confirm the absence or presence of coliforms or E. Coli. Enumeration can be undertaken with Colilert and Colilert 18 using a Quanti-Tray system in a laboratory.

Given that Coliforms and E Coli double every 17 - 30 minutes, the time taken for the coliforms and E. coli to metabolise the ONPG and MUG to show the yellow colour or fluorescence is indicative of the total number of colony forming units (CFUs).

## The device

The device includes:

* A dry block incubator
* A white light (which could be an LED).
* A light (which could be an LED) that emits ultra violet light at the 365nm wavelength.
* A Red Green Yellow colour sensor;
* An RFID reader and writer (optional); and
* A microcontroller.

The device is thermally insulated to retain heat.

## The process

The operator adds coliform enzyme β-galactosidase and the E. coli enzyme β-glucuronidase to the water sample replaces the and shakes the sample until the media is dissolved.

The Sample is placed in the dry block incubator.

Once the device is powered on, it checks that:

* There is no water present in the USB socket
* There is sufficient current (amps) from the 5v USB power supply.

The operator is alerted if there is a problem.

Operator starts the process by pressing start button.

The device checks that the RFID tag on the sample container is authentic (check encrypted hash value). If the RFID tag is verified and there is no data on the tag indicating that processing has already been attempted, then the process continues. Otherwise, the operator is alerted to the problem and the process stops.

The current temperature of the sample is estimated by measuring the temperature of the dry block after the sample is placed in the dry block.

The dry block is heated at 44.5 degrees for a duration. The duration is calculated by;

Once the sample is at the estimated temperature of 35 degrees Celsius, the dry block will be heated at a constant temperature of 35 degrees +/- 0.5 degrees. The temperature range considers the accuracy of the temperature sensor. For example, a platinum temperature sensor may have an accuracy of +/- 0.35 degrees at 35 degrees so the temperature must be maintained within the range of 34.85 – 35.15 degrees. Pulse-width modulation can be used to control the temperature.

The sample will be illuminated with a white light at regular intervals (for example, every minute). While the sample is illuminated, the RGB colour sensor will measure the colour of the sample. As the incubation process continues and coliforms are present, the water sample will gradually turn yellow. The RBG values of the sample will be compared to the RGB values of the test’s Comparator. The test will be positive for Total Coliforms if the RGB colour is of an equal or darker yellow than the test’s comparator. What is test’s comparator? What happens after test is positive do I have to perform this test again until test time ends?

The sample will be illuminated with a UV light using the 365nm wavelength at regular intervals (for example, every minute). While the sample is illuminated, the RGB colour sensor will measure the colour of the sample. As the incubation process continues and coliforms are present, the water sample will start to fluoresce under the UV light. The number of CFUs will be estimated time from the start of incubation to the time that the RBG values of the sample approximately match the RGB values of the reagent’s Comparator. What is regent’s comparator? What happens after test is positive do I have to perform this test again until test time ends? I did not understand number of CFUs?

If after the maximum test time (for example, 16 hours for Colitag or 24 hours Colilert), any yellow colour is lighter than the Comparator then the sample is negative for both Total Coliforms and E. Coli. When you say any colour, you mean when test perform during illuminated white/UV light. right?

If after the maximum test time, any fluorescence is less than the comparator then the sample is negative for E. Coli.

If the colour sensor detects light when the White or UVA LED are not illuminated, the operator will be alerted that the incubator lid is open. No results from the colour sensor will be used until the external light is no longer detected.

If the temperature sensor detects that the temperature is outside of the acceptable range (for example, 35 degrees +/- 0.5 degrees), then the operator will be alerted and the Sample will be rejected from the test. Alerted How?

The sample will be inactivated after the test is completed by illuminating the sample with UV-A light and a dosage of 250mJ/cm2. Assuming a 44mm x 80mm sample cylindrical container, the surface area is expected to be 140.99 cm2. With a minimum radiant flux of 850mW from a UVA LED, the sample should be inactivated (1 log) in 41.5 seconds.

Results of the tests can be transmitted from the device to a website or to a mobile phone. Do you have any mobile app or website?

Every six months the PT100 sensor must be calibrated using an ice point method. This method involves the dry block being filled almost to the top with crushed ice and then adding water. Within two minutes the temperature should read 0 degrees. If the temperature is more than +/- 0.5 degrees then the unit must be sent for servicing. The PT100 calibration offset must be updated after the two-minute period. A calibration record will be saved to a log on the device. The calibration record will be transmitted from the device to a centralised database.

# Dry block

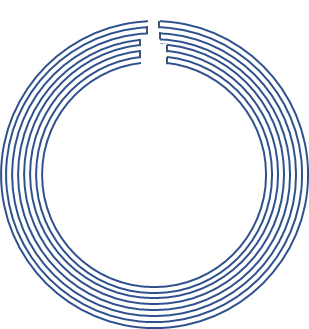
The dry block is made from material with a high degree of thermal conductivity (such as 6063 Aluminum).

The interior sides of the dry block are angled to match the angles of the sample container. There is a small channel from the top to the bottom of the interior of the dry block to allow air to enter and exit the dry block as sample container enters and exits the dry block. This avoids the sample being stuck in the dry block.

The LEDs and colour sensor are separated from the sample with a UV transmissible material such as quartz or UVT grade acrylic.

### Heating

The PCB will contain a heating pad with a design similar to the one below. The PCB will be circle shaped and have a diameter of 54mm.



The PCB track will have sufficient resistance to heat up it to 44.5 degrees with 5v power supply.

Aluminium block will be fitted to a heating pad using thermally conductive, electrically insulated adhesive.

Temperature to be controlled by PT100 RTD using PWM. The PT100 SMD component will be located in the middle of the heating element. A small recess in the Aluminium block will allow the PT100 0805 SMD component to sit inside the aluminium block.

The Base PCB will contain an amplifier for the PT100 sensor.

## Colour sensor

The colour sensor comprises of several photodiodes with RGB filters attached to it. Other filters may also be included in the sensor.

The colour sensor is shrouded by a structure that reduces the light being emitted from the UVA and White LEDs being detected by the colour sensor. This shroud focuses the colour sensor on the sample container.

## White LED

A white SMD LED (and resistor) is located near the centre of the PCB. This LED is turned on to illuminate the sample container using a microcontroller.

## UVA LED

A SMD LED with a peak wavelength of 365nm (and resistor) is located near the centre of the PCB. This LED is turned on to illuminate the sample container using a microcontroller.

## Underside centre of PCB must allow for suction cup

The PCB will be handled in the assembly process using a suction cup attached to the underside. The 20mm centre underside of the PCB must be flat and have no holes.

## Alignment notches

The PCB shall have 3 notches on its perimeter to assist with the alignment of the PCB during the assembly process.

# Sample Containers

The sample containers are clear and made of a non-fluorescing material (such as polystyrene). The sample container must be sterilised before use (for example, by using gamma radiation or Ethylene Oxide at the manufacturing facility).

The sample container may have angled sides so that the sides of the sample container are in contact with the dry block. This contact helps to transfer the heat from the dry block to the sample.

## Microcontroller

The microcontroller must be able to:

* Be updateable over the air (OTA).
* Include WiFi and BLE communications.
* Perform the functions described in this document.

An ESP32 microcontroller is assumed.

The device must only be updated with code from the manufacturer.

## RFID

An RFID antenna is located directly above the sample container when the lid is in the closed position. This RFID reader/Writer will be used to:

Read data from the RFID tag such as:

* the serial number of the RFID tag;
* data added by the mobile app during the sample collection process such as
  + the type of sample (treatment system, distribution zone, raw water)
  + date and time that sample was taken
  + method of collection,
  + name of sampler (person)
  + location (Longitude/Latitude)

Write data to the RFID tag such as:

* Process step and the time of the step
* Testing product being used (eg Colilert, Colilert 18, Colisure, Colitag).
* Failure codes.

### Power button

A power button turns on the device. Holding the power button down for 5 seconds starts a Bluetooth pairing process.

# Additional requirements

## Waterproof external USB socket

The USB socket must be IPX4 rated. This will protect water ingress during the Ice-point calibration.

## Battery backup

The device has a battery backup that will allow it to operate without power for 15 mins.

## Power filter

The device has a power filter to protect it from surges which could be caused by diesel generator power sources.

# Mobile App Requirements

## Sample collection

* Step by step guide. (eg flame or alcohol swab faucet, run water for 6 mins, collect sample)
* Advise if sample has been collected in the location many times before (distribution Zone samples).
* Collect user, date/time, location, type of sample data.
* Write data to the RFID tag

## Send data to server

The mobile app must be able to transmit data collected by the device and the sample collection process to a cloud service.

## Setup incubator

The app will be able to setup and maintain the device . These functions include:

* Connect to wifi (if available)
* Calibrate temperature using the ice-point method.
* Setup other communication devices (eg Bluetooth link to satellite modem)
* Update firmware on Device

## Reports

The app will display information collected by the device. The user will be able to:

* View test results by table and map.
* Search for test results
* Share reports with users
* Share report with regulators

# Enclosure requirements

The enclosure will:

* Be made from robust polymers and will have UV and microbial protection additives.
* be able to be cleaned with sterilising cleaners such as bleach and alcohol.
* have rounded corners for drop protection
* be filled with insulation material such as high-density polyurethane foam.