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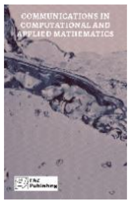

Portable Incubator For E.coli and Coliform Bacterial Growth Using IoT

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Abstract: Clean water is essential to healthy lifestyles. This is because when E.coli and Coliform are in the water it indicates that pathogens and parasites have contaminated the water. Every day microbiologists in Malaysia monitor water quality to ensure that consumers obtain clean water. This portable incubator is therefore important for microbiologists to carry out inspections of water quality everywhere and does not necessarily make observations only in the Microbiology laboratory. The identification of E.coli and Coliform bacteria is achieved using both qualitative and quantitative approaches. Detection was achieved for the qualitative process by injection of the reagent into a water sample using a sterile bottle. While for quantitative methods, the Quanti-tray used reagents to detect the presence of E.coli and Coliform, calculate the Most Probable Number (MPN) and 95 % Confidence Limit in a water sample. In addition, these innovations can also perform the incubation process manually. RoboRealm was used to identify E. coli and coliform for both methods based on color changes. The water sample used for this research was collected in two areas in the district of Johor, Kluang and Batu Pahat. Using computer vision and Internet of Things (IoT), two datasets containing more than 120 images have been used for the evaluation process and 30 images were randomly selected for calibration process. This innovation shortens the time for detecting the presence of E.coli and Coliform bacteria in just 2 hours, result can be viewed in real-time and the implementation of security two-factor authentication (2FA).

Keywords: E.coli, Coliform, IoT, MPN, portable

1. Introduction

A prime source of pathogens in water is from animal and human fecal contamination that poses a significant health risk for millions of people worldwide [1,2]. Although there are several barriers to determining water quality [3], water quality testing for bacteria in water is crucial as outlined by the World Health Organization (WHO) to ensure safe drinking water to consumers. Escherichia coli are the most widely used water safety microbial indicators alongside other coliform group members [2]. Thus, this portable incubator was developed to detect E.coli and Coliform bacteria in treated or contaminated water sources. The idea for this innovation was sparked by reading the concerns of the microbiologist to test the water samples in the water source. Traditional methods for water quality testing are usually performed only in the laboratory. The advantage of this incubator is that it can use a direct current (DC) connection by using a battery source. Thus testing to detect the presence of E.coli and Coliform bacteria can be done anywhere. This is because, to see if the E.coli or Coliform bacteria are present in the water sample it takes at least 18 to 24 hours. The incubator also required an optimum temperature of 37 ° C ± 1 ° C to allow the water sample to react with the reagent for detection of E.coli and Coliform in water. A robust field incubator 's reliability lies in its ability to reach and maintain a set temperature under different conditions [4]. This process is called bacterial growth [5]. Using this innovation,

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the time to detect the presence of E.coli and Coliform bacteria can be shortened. Through a study conducted in conjunction with Ranhill SAJ at the SAJ Sri Gading Central Laboratory, this incubator was able to detect the presence of E.coli and Coliform bacteria in just 6 hours. In addition, the incubator utilizes image processing technology and the Internet of Things (IoT), both of which are important elements of the Fourth Industrial Revolution (IR4.0). Both Quantitative and Qualitative methods can be done using image processing methods. Additionally, these innovations can also manually execute the incubation process by inserting the value of incubation time and sampling time. RoboRealm software is used to process water sample datasets for both methods based on color changes in the water sample. If the water sample is yellow and thicker than the comparison, therefore this sample is Coliform positive and if the sample is luminous, then the water sample is E.coli positive. On this incubator, LattePanda is used as single board computers controlling all inputs and outputs. LattePanda is connected to the screen so the user is able to observe the incubation process. This incubator is also connected to a mobile phone equipped with QR Water apps which enables it to input the collected water sample data as well as see MPN values and observations in the incubator in real-time. These QR water apps were developed using Kodular software [6]. This QR Water function is also connected to a QR code scanner where it can print the QR code that has been created. This QR code will then be pasted on a sterile bottle or on the Quanti-tray. This QR Water apps uses Two-factor authentication (2FA) technology, where only users registered in the database can use this QR Water apps and can control the functionality of the incubator. The user must enter the correct password on the first layer of security, and if appropriate then the user will go through the second layer of security by scanning the fingerprint on the mobile phone. Additionally, while making this innovation user-friendly, Microsoft Visual Studio software is used to create the Graphical User Interface (GUI) for viewing on a computer. GUI makes it easy for users to select item type, incubate parameters, incubate analysis, I / O control and setting for Kodular applications (handphone control) and serial setting (LattePanda).

2. Research Method

This incubator making process involves two main components: software and hardware.

There are four main elements for the software components:

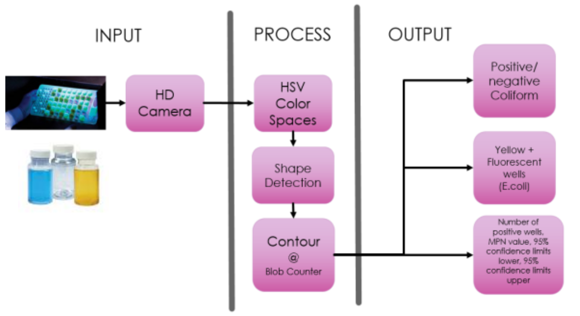
a. Computer vision using RoboRealm
b. Kodular to build the QR apps
c. GUI for computers using Visual Studio and the Bunifu plugin
d. Arduino for controlling lamps and UV lamps

For hardware components, the three elements are:

a. Portable incubator
b. Sealer machine for Quanti-tray
c. DC battery

2.1. Computer vision

A key component of the development of this incubator is the detection of E.coli and Coliform bacteria by using recorded images. The RoboRealm software is used to process water sample images in this incubator. RoboRealm allows users with advanced modules such as the agnostic camera and multiple image sources to design prototypes. The use of Roborealm [7] makes vision programming easier. Figure 1 shows a block diagram for the RoboRealm vision process. The HD camera captures the sterile bottle and the Quanti-tray, and then deals with HSV color spaces to read raw images with the program of image processing and convert images into different color spaces to gather information on the various color channels [8], shape detection in order to prevent color failure, detect and analyze the right sample of Quanti-tray with a square array [9] and blob contour by controlling color, proximity, location, size, texture, shape, moment of inertia, edge, orientation and other variables to make image detection more precise [10] This method will generate 3 outputs, namely positive / negative Coliform, positive / negative E.coli, MPN values and 95% upper and lower confidence limit.



The incubator needs to be calibrated before it can be used for the purpose of research. The process of calibration is carried out by a high resolution camera and RoboRealm software. This calibration process is done to ensure that the camera captures the image clearly and the camera must be reset when the image is blurred and not sharp. In order for the RoboRealm program to process the picture, the images taken by the camera must be clear. A tiny mobile monitor for record the image and observing color change in the water samples is connected to the incubator as shown in Figure 2.

Figure 2: Image calibration using RoboRealm

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2.2 QR apps

QR apps are an application built with Kodular software on mobile phones [11]. The apps are available on Android OS-based mobile phones. There are 3 screens built using Kodular. Screen 1 is a "Welcome Screen" which is where the user enters the username and password as shown in Figure 3. This screen 1 also shows the current date and time.

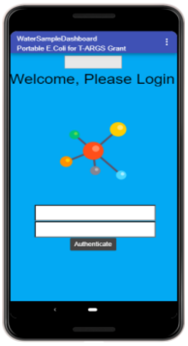

Figure 3: Welcome screen

Screen 2 acts as a loading screen as shown in figure 4. A loading scene is a visual placeholder shown to the user while the app loads.

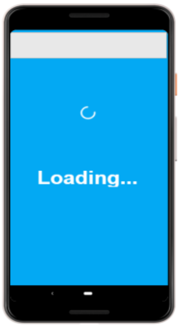

Figure 4: Loading screen

Figure 5 shows the main screen which is the third screen in the Kodular. This screen displays MPN value, 95% confidence limit for upper limit (UL) and lower limit (LL). This screen also provides functionality to generate stickers and live monitoring incubator conditions.

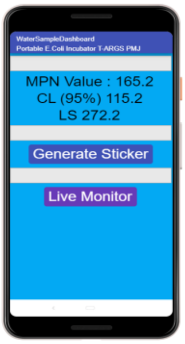

Figure 5: Main screen

The three main menus at Kodular are Assets, Designer and Blocks. Assets are the user's place for storing images to be placed on the screen, while the designer menu is for the user to design the GUI. Blocks as shown in Figure 6 are the place for the user drag-and-drop desired functionality.

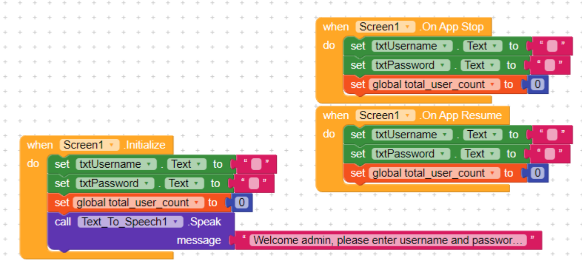

Figure 6: Blocks menu in Kodular

2.3 GUI for PMJ E.coli PI apps

The graphical user interface (GUI) is a user interface that permits users to use graphical icons and the audio symbol instead of text-based user interfaces, typed command labels or text navigation to communicate with electrical devices. The GUI is user-friendlier compared with command-line interfaces (CLIs), which require a user to enter commands on a device. According to [12], the advantage of GUI is that it enables the user to work faster, higher productivity and increase the user's desire to explore the system.This GUI for computers was designed using the Visual Studio and the Bunifu plugin.

This portable incubator's GUI layout consists of 6 main menus, and the first menu is sign in to enter username and password, as shown in Figure 7. GUI shapes desktop applications known as PMJ E.coli PI apps. PMJ E.coli PI apps on this incubator is protected by username and password to prevent unauthorized persons from accessing the system. The Login Success pop-up window will appear as displayed in Figure 8 if the user enteres the correct user name and password.Login Failed! pop-up window instantly appears when a user enters an incorrect password or username such as Figure 9. The user needs to click on Close button if the user wants to sign off this system.

Figure 7: Sign in menu

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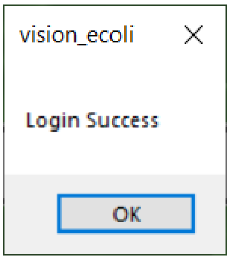

Figure 8: Login Success pop-up window

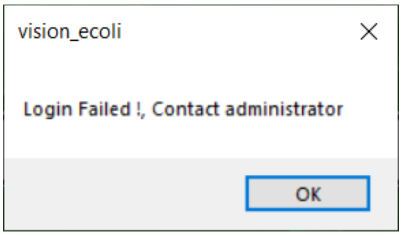

Figure 9: Login Failed! pop-up window

Once the correct username and password are successfully entered, the user moves to a second screen. As illustrated in Figure 10, this screen has a menu in which the user can select either the sterile bottle or the Quanti-tray which is known as the Colilert-18 mode in these applications. Furthermore, the manual method is the third choice of this applications. This manual method can be used if the user is not using a sterile bottle or the Quanti-tray.

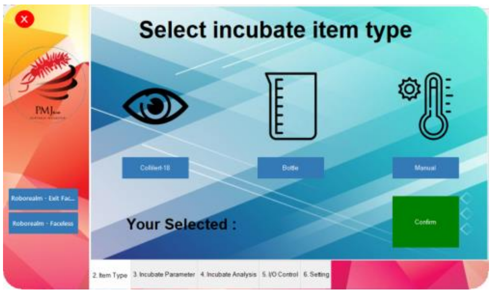

Figure 10: Selection screen

Figure 11 shows when the user selects the Quanti-tray method.

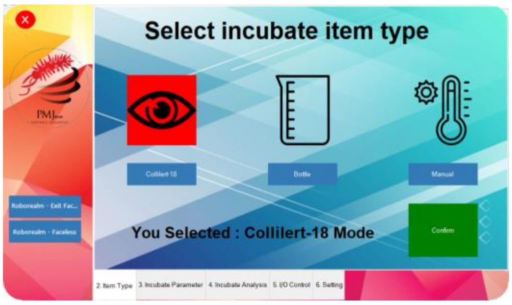

Figure 11: Collilert-18 mode

Once a user chooses the Collilert-18 mode, a popup window for the user will appear to proceed to the next phase or to terminate the process, as shown in Figure 12.

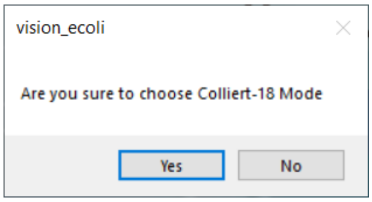

Figure 12: Collilert-18 mode pop-up window

The setup process for incubation is shown in Figure 13. Incubation time and sampling time are the parameters involved. Incubation time is the time required for the growth of the E.coli and Colifom bacteria. Sampling time refers to the time set by the user for camera to capture the water sample on the incubator.

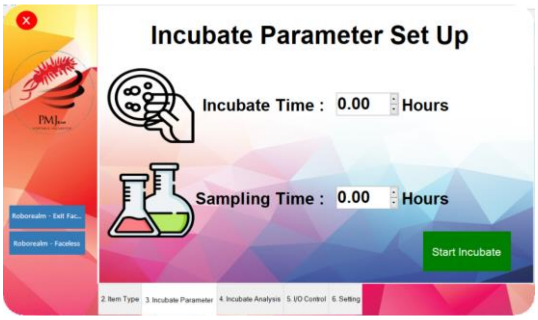

Figure 13: Setup parameter

The fourth menu is a process of incubation analysis which is divided into several other menu tabs: Summary, Temperature, Humidity and vision. Summary screen as shown in Figure 14 will display three displays: the remaining time that the user has set in the previous menu and the percentage of brightness for light bulbs and fluorescent lamp.

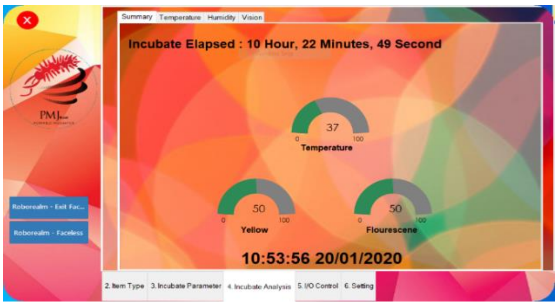

Figure 14: Incubation analysis

The current value in the ampere unit (A) is shown in the sine wave for the temperature and humidity tabs as shown in Figure 15. When the sine wave is formed, this indicates that there is a current flowing between the connection of main board and DHT22 sensor.

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Figure 15: Sine wave for temperature and humidity

Figure 16 shows the image that the camera has captured of the equipment included in the incubator. This enables users to see the growth of E.coli and Coliform without opening the incubator door. As explained in [13] it affects temperature and humidity within the incubator if the incubator door is opened regularly.

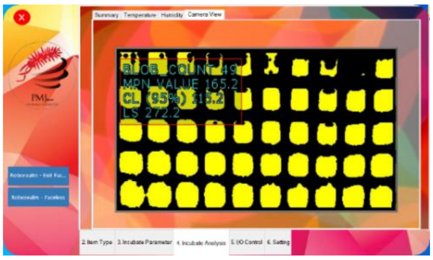

Figure 16: Camera view

LED and UV lamp controls are shown in Figure 17. Through using the button led enable or UV enable, users can either turn on or off. It helps the user to conserve electricity (if the user is in the ac mode) and save battery cells (if the user is in the dc mode).

Figure 17: Controlling LED and UV lamp

The settings for Firebase are shown in Figure 18. This setting is required in order to obtain mobile device data readings. Data using this firebase feature is stored in the cloud and is JSON-friendly.

Figure 18: Firebase setting

The final menu is the serial setting. Once the user clicks on the Connect button, these PMJ E.coli PI apps will communicate with LattePanda which has Arduino based. This display shows the communication port and temperature value used, as shown in Figure 19.

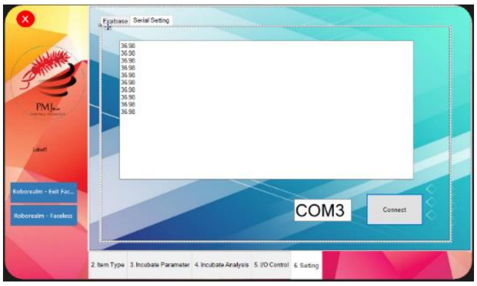

Figure 19: Serial setting

2.4 Arduino

The program Arduino is used since the design of the LattePanda is Arduino compatible. LattePanda pins are connected with sensors, LEDs and UV lamps. This sensor DHT22 is used to detect changes in temperature and humidity. The following is the pseudocode for the DHT22 sensor.

```
DHT22 pseudocode
  Program start
  Creates a DHT object
  Start infinite loop
    Reads the data from the sensor
    Get the values of the temperature
    Get the values of the humidity
    Printing the result on LCD display
    Call function delay for 5 seconds
  End infinite loop
```

Outputs are LEDs and UV lamps connected to the relays. Below is an example of the pseudocode for controlling LED and UV lamp using PMJ E.coli PI apps.

```
Controlling LED and UV lamp pseudocode
  Program start
  Include FirebaseArduino library
  Initialize Firebase Host and Auth
  Initialize Wifi SSID and password
  Initialize relay
  Start Firebasereconnect
  Start infinite loop
    Reading the value of the variable Status
    from the firebase
    Status 1=Turn on the relay
    Status 2=Turn off the relay
  End infinite loop
```

2.5 Portable incubator

The front view is shown in Figure 20 while the side view for a portable incubator is in Figure 21. The first switch is used to activate or deactivate the AC connection, while the other two switches are used to manually turn on or off the LED and UV lamp.

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(a) Front view (b) side view

Figure 20: Incubator from front and side view

Figure 21: Incubator display and switches panel

The high-resolution camera in an incubator to capture images for the identification of E.coli and Coliform bacteria is shown in Figure 22.

Figure 22: High-resolution camera

Figure 23: Sterile bottle container

Figure 24 shows the container for placing a water sample using a sterile bottle technique. This container can accommodate up to 12 units of sterile bottle and is designed to allow water samples to go through the incubation process everywhere without any spillage. The Quanti-tray slot are shown in Figure 24.

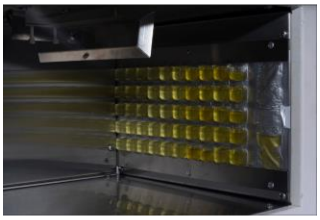

Figure 24: Quantitray slot

2.6 Sealer machine

Figure 26 shows the sealer machine from the front view and Figure 27 shows the sealer machine when the molding door opens. This sealer machine uses AC supply mode. The mold on this sealer is used for sealing the Quanti-tray. Figure 28 shows the display and switch panel for turn on or off the AC supply. The user has to wait until 80^{o}C temperature to get the perfect sealing. The leak occurs in Quanti-tray when the user proceeds with the sealing process under 80^{o}C.

Figure 25: Sealer machine front view

Figure 26: Quanti-tray

Figure 27: Sealer machine display and switch panel

2.7 DC supply

Figure 29 shows the case for DC supply. Inside the case is a car battery and a 12V / 24V 400Ah car battery charger as shown in Figure 30. This car battery acts as a supply current to allow the incubator to operate and last for 24 hours. When the battery starts running low, it will be recharged using a battery charger. In addition to the recharge process, the battery charger can perform repairs and maintenance as well as have intelligent battery charger function will automatically stop charging when fully charged. Figure 31 shows the holder on this DC supply case to allow the user to easily lift it.

Figure 28: DC supply case

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Figure 29: Car battery and battery charger

Figure 30: Holder for DC supply case

3. Result and Discussion

The results of this study are divided into two methods that is qualitative method using sterile bottle and quantitative method using Quanti-tray. Table 1 shows the number of water samples and types of water used for the qualitative and quantitative methods. A total of 120 water samples were used for this experiment, of which 30 samples were randomly selected for calibration purposes.

Table 1. Table method vs type and number of water samples

Method	Treated water Zone A	Unreated water Zone A	Treated water Zone B	Unreated water Zone B
Qualitative	15	15	15	15
Quantitative	15	15	15	15

Table 1 shows 30 samples of treated water taken for the purposes of this study in Zones A and B for both qualitative and quantitative methods. This treated sample was collected from two water sources in the Ranhill SAJ dam in the district of Batu Pahat, for Zone A and Zone B in the district of Kluang. Water samples were taken in river areas around Batu Pahat for Area A and Kluang for Area B from unprocessed water sources. A sterile bottle containing sodium thiosulfate was used in the quality method, and a bottle without sodium thiosulfate was used for untreated water. As shown in Figure 32, water samples collected in these two areas are mixed with the Colilert-18 reagent.

Figure 31: Colilert-18 reagent

Colilert-18 is used as a reagent as it contains Defined Substrate Technology (DST) nutrient indicator ONPG and MUG to detect the presence of E.coli and Coliform bacteria [14]. This reagent can produce results as early as 18 hours and is also approved by the U.S. EPA as a standard method for examination of treated and contaminated water. According to [15], using this reagent can achieve 100% test accuracy. Water samples mixed with the Colilert-18 reagent will be shaken to ensure that the reagent is completely soluble in water. If using the Quanti-tray technique, the water sample will be shaken first using a sterile bottle and placed into the Quanti-tray. The Quanti-tray will next be sealed by a sealer machine. A 1-hour photo of the water sample is taken to see the colors change as shown in Figure 33 using Quanti-tray technique in order to find the number of hours required to detect E.coli and Coliform bacteria.

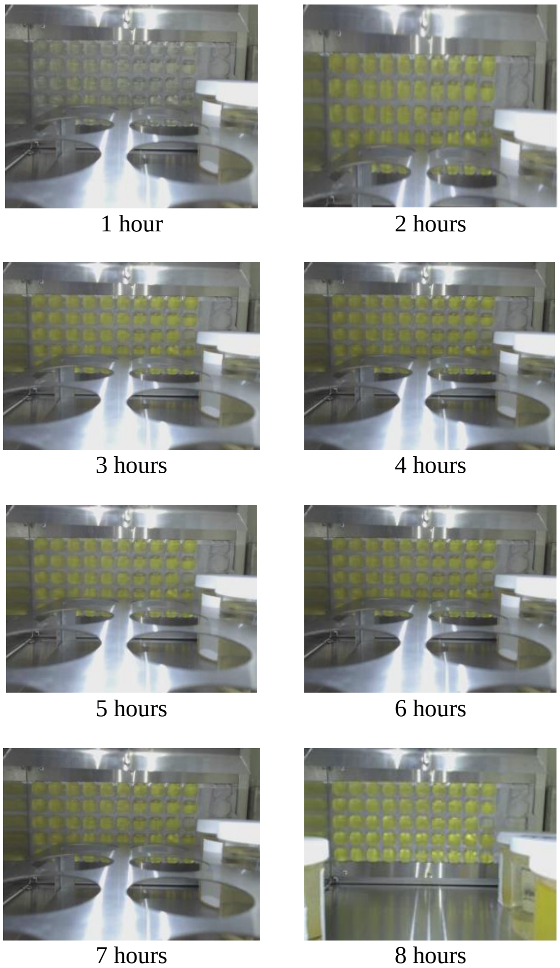

Figure 32: Hourly observations

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LattePanda is set to take pictures every 1 hour. A comparator was used to test each sample visually for the existence of Coliform bacteria [16]. Figure 35 shows the bottle comparator and Figure 36 shows the Quanti-tray comparator.

Figure 33: Bottle comparator

Figure 34: Quanti-tray comparator

The observations show the water sample color began to change to a yellow color in the first hour. The water sample 's color had turned yellow by the second hour. The first two hours of change in color are identical to the result of 8 hours. PMJ E.coli PI apps with RoboRealm will examine these bacteria Coliform on the basis of hourly images. The pictures taken are compared with the Quanti-tray comparator. This sample contains coliform bacteria if the color of the water sample is more yellow than the comparator. E.coli bacteria were later identified using Fluorescent 365 nm lamps. This sample of water is positive for E. coli if the water sample is luminous. PMJ E.coli PI apps display positive E.coli values, MPN values and 95% upper and lower confidence limit as shown in Figure 37. The use of this technology allows for automatic computation of the MPN values and 95% confidence limit without the need for users to use the IDEXX 51-Well QuantiTray table as shown in Figure 38. This saves users time and makes things easier.

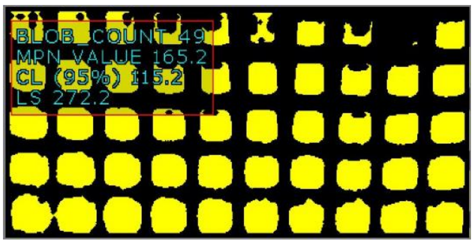

Figure 35: Quanti-tray display on PMJ E.coli PI apps

IDEXX
51-Well Quanti-Tray®
MPN Table

No. of wells giving positive reaction	MPN per 100 ml sample	95% Confidence Limits Lower	Upper
0	<1.0	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	> 200.5	146.1	infinite

IDEXX Sales and Technical Support
1-800-321-0207 or 1-207-856-0496
www.idexx.com/water

Figure 36: IDEXX 51-Well Quanti-tray table

The image of water samples using the sterile bottle that are included in the incubator will also be taken every 1 hour. The picture taken is compared to the bottle comparator. If the color is thicker then the water sample is Coliform postive and if it glows when placed under the Fluorescent 365nm lamp as shown in Figure 39, then this water sample is E.coli positive. Figure 40 shows Coliform positive and Figure 41 shows E.coli positive using PMJ E.coli PI apps.

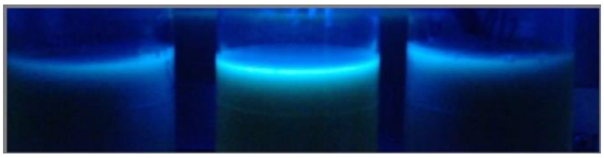

Figure 37: Illuminated water sample using 365nm Fluorescent lamp

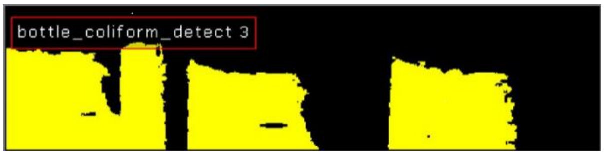

Figure 38: Coliform positive

Figure 39: E.coli positive

Based on the graph in Figure 42, the results of using the bottle and quanti-tray method for treated water in Zone A and Zone B yield 100% negative E.coli and Coliform bacteria. 80% samples positive E.coli and Coliform bacteria when used untreated water for both bottle and quanti-tray method for Zone A . Whereas for untreated water samples in Zone B produced 67% positive E.coli and Coliform bacteria.

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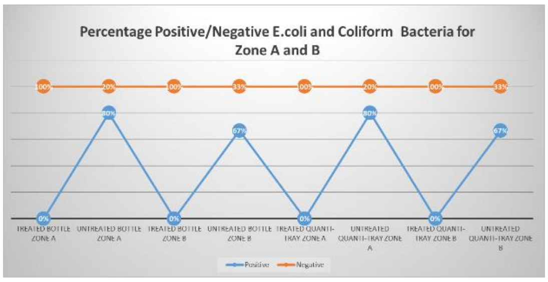

Figure 40: Percentage of positive and negative

4. Conclusion

This research demonstrated that the goal was to build a portable incubator which uses vision and IoT to detect E.coli and coliform bacteria. E.coli and Coliform bacteria can be detected with this incubator for a minimum of 2 hours. High-speed imaging technology can save 90% of user time compared to traditionally employed methods. In addition, result and data are in real time. This incubator design is compact and mobility. The incubator operates in two methods, using sterile bottle and Quanti-tray. The sterile bottle method is used to determine whether or not Coliform and E.coli bacteria are present. The Quanti-tray method, however, is also used to detect the presence of E.coli and Coliform bacteria and provides MPN readings and a 95% upper and lower confidence limit. Thus, it saves users time as users no longer need to use the IDEXX 51-Well Quanti-tray table. Verification has also been done by comparing this result incubator with the incubator used by Ranhill SAJ. 60 water samples were used by this incubator as well as 60 water samples were used by the Ranhill SAJ incubator. Comparative techniques between these incubators are necessary because the incubators at Ranhill SAJ meet the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality. The results produced by this incubator were identical and successfully detected 100% detection of E.coli and Coliform bacteria. Wireless monitoring is used to allow the user to monitor the situation inside the incubator anywhere. In addition, this innovation also uses two factor authentication (2FA) to protect the system from outsiders. For future development, the preferred mode according to the type of bacteria in the water can be added such as the bacteria Yersinia, Salmonella and Shigella.

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