

DEVELOPMENT AND FABRICATION OF AUTOMATED MEDIA PREPARING MACHINE

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Technology degree in Biotechnology
Interdisciplinary project

By

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DEPARTMENT OF BIOTECHNOLOGY

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**SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY**

(DEEMED TO BE UNIVERSITY)

Accredited with grade “A” by NAAC

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MAY 2022



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Sandhya .S (Reg. No. 39230037), Britlin Deva Jebasta .N (Reg. No. 39230008), Rakshi .A.D (Reg. No. 39230035), and Raagav S R (Reg.No.39090040) who carried out the Interdisciplinary Project entitled “**DEVELOPMENT AND FABRICATION OF AUTOMATED MEDIA PREPARING MACHINE**” under our supervision from February 2022 to April 2022.

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I, Sandhya .S (Reg. No. 39230037) hereby declare that the Project Report entitled **“DEVELOPMENT AND FABRICATION OF AUTOMATED MEDIA PREPARING MACHINE”** done by me under the guidance of **Dr. INBATHAMIZH L, M.Sc., Ph.D.** and **Dr. LILLY MERCY J, M.E., Ph.D.** is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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AKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of **SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my sincere thanks to **Dr.Y.Swarnalatha, M.Sc., Ph.D.**, Dean, Higher Studies and **Dr.V.Ramesh Kumar, M.Phil., Ph.D.**, Head of the Department, Department of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my internal Guide **Dr. INBATHAMIZH L, M.Sc., Ph.D.** and external Guide **Dr. LILLY MERCY J, M.E., Ph.D.** for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I also express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project work.

ABSTRACT

Microorganisms are almost widespread, numerous, and indispensable for human life. One of the prerequisites for studying them is the preparation of standard culture media. Microorganisms flourish in a range of habitats and have various developmental needs, such as nutrition, pH, osmotic conditions, and temperature. The production and delivery of culture medium are one of the most time-consuming and tedious operations in the modern microbiology laboratory. Despite the fact that many laboratories have improved their testing procedures and methodologies, most laboratories still use stir plates, flasks, autoclaves, and peristaltic pumps to prepare and dispense media. This manual preparation technique increases the potential of media contamination and poses a safety risk to the personnel who prepare the media (transferring heavy, hot flasks from the autoclave to the benchtop). There are now reliable solutions to these process challenges in the form of high-capacity automated media sterilizers and plate pourers. We built a fully automated unit that prepares culture media formulations in independent culture in a sterilized environment. The media preparation system will automatically mix, sterilize and automatically dispense the proper volume into the sample container quickly and accurately. Laboratories that use this automated technology will see considerable improvements in media quality and production efficiency, as well as significant labor savings. These instruments aid in increasing productivity, operator safety, and media quality. These automated media preparation and dispensing systems are becoming a need for the modern effective microbiological testing laboratory, as the demand to do more with fewer increases.

Keywords: Media preparation, Automation, Dispenser, Sterilization.

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LIST OF SYMBOLS AND ABBEREVIATIONS

FDG	F-fluorodeoxyglucose
EANM	European Association of Nuclear Medicine
PET	Positron Emission Tomography
UV	Ultra Violet
AC	Alternating Current
DC	Direct Current
PV	Photovoltaic
SPM	Special Purpose Machine
LED	Light-Emitting Diode
NA	Nutrient Agar
PDA	Potato Dextrose Agar
ICAR	The Indian Council of Agricultural Research
ICMR	The Indian Council of Medical Research

1. INTRODUCTION:

Microorganisms are nearly ubiquitous, abundant, and necessary for human survival. Preparation of standard culture medium is one of the conditions for researching them. One of the most time-consuming and tiresome processes in the contemporary microbiology laboratory is the preparation and distribution of culture medium, which poses a safety risk to the workers who prepare the media (transferring heavy, hot flasks from the autoclave to the benchtop). In the shape of high-capacity automated media sterilizers and plate pourers, there are now viable solutions to these process difficulties. In a sterile environment, we created a completely automated device that creates culture medium formulations in independent culture. The media preparation system will mix, sanitize, and dispense the correct volume into the sample container automatically. The media preparation system will rapidly and correctly mix, sterilize, and dispense the appropriate volume into the sample container.

Automated media preparers will prepare, sterilize, and distribute liquid or solid culture medium automatically. Preparing and distributing plated medium or broths in tubes and bottles in a busy laboratory can be time-intensive, especially when large sample numbers need to be processed. Although lower labor costs are a compelling reason to implement an automated media preparation process, there are other considerations to consider: the cost of storage space and facilities if ready-to-use [RTU] media is used [plus, of course, the purchase price]; manual media preparation can be prone to contamination. For those times when the typical laboratory workflow is disrupted, an in-house automated media preparation system can provide a virtual on-demand supply of media. At its most basic level, media preparation automation might entail the repeatable transfer of liquid agar or broth to plates, tubes, or bottles. For smaller laboratories, semi-automated filling systems can do this. When producing huge quantities of plates, tubes, vials, bottles, and other items, the entire process can be automated. High-capacity fully automated media sterilizers, plate pourers, fillers, and dispensers are now available. Simply add dehydrated medium, pick the appropriate process protocol, and wait for the pre-poured plates, tubes, or other items to arrive. Alternatively, because such systems are walk-away, you may go out and do something else. These microprocessor-controlled fully automated systems provide you complete control over process parameters including sterilization temperature and duration, pouring temperature, and so on. At certain phases in the process, certain systems can introduce thermolabile materials like blood or selective agents. With certain systems, the entire procedure may be monitored by computer, allowing for comprehensive batch traceability. Plates, for example, can be automatically bar tagged with the relevant batch or lot number.

The media preparation procedure does not have to be completely automated in all laboratories. Almost all microbiology labs will have access to or already have an

autoclave or sterilizer; if this is the case, all that is required of many laboratories is an automatic dish stacker/pourer or a bottle/vial dispenser. Some of them, like fully automated systems, can have optional printers for plate identification and other purposes. Accessories that can pour bi-plates might be included as well. Non-standard plate designs or wholly other formats, such as microtiter plates, may need to be filled with agar, broths, or other liquid on rare occasions. In most cases, a robotic system may be used to alleviate these problems, allowing for a genuine "walk away" operation once again. These can even automate the entire sample assay depending on the accessories and sample/assay in question. This sort of system is often used in clinical settings and is normally referred to as an integrated sample assay device rather than a robot.

2. REVIEW OF LITERATURE:

2.1. PERCEPTION IN ROBOTICS:

Robotics has come a long way during the development of our country. To advance further in robotics we need to improve sensing to make robots more user-friendly and capable to do out the work for us automatically by just giving it an input (Sanders, 1999). The performance of silicon microchip processors and memory is still doubling every 18 months. High-end processors can run at 750 MHz with four million transistors per chip. By 2003, they are expected to be running at 1500 MHz with 18 million transistors per chip. New processors may have between 5 and 20 clock instructions per clock cycle, which is the equivalent of 10 billion instructions per second per chip!

As computer processing increases in power so robots may improve their perception and learn to use new tools and sensors. Human implants may also become more common and humans may communicate directly with external sensors or machines through other means. Computer scientists and software engineers are making rapid progress with research in object-oriented languages and parallel processing. Although the code produced by these languages may sometimes be less efficient than hard-coding, it is generally more flexible. The idea of parallel inheritance from existing classes to new classes may be powerful for the control and understanding of robots and for how sensors are treated and integrated into systems. In any case, the new way of thinking involved in this will lead to new methods and ideas for the simple integration and representation of information from sensor data.

If we can achieve this integration then many of the problems we think of today as high-level problems may just become low-level nuisances. For example, dual robot coordination may become simple. Work may move to a higher level to concentrate on the application or the task. A multitude of robots may be treated as one entity. In image processing, the recognition and positioning of different objects are becoming easier, so that systems can concentrate on what they are going to do with objects rather than what they are and where they are. That is, to concentrate on the task rather than the parts.

All this depends not only on improving sensing to make robots more user-friendly and capable but also on the ability of the robot to understand tasks in real-time so that they know what is happening as it happens. This includes advances in modeling tasks and the environment and the integration of the two to generate new algorithms. These algorithms can be used for reasoning about relationships between objects and tasks and for inferring the results of possible actions. If sensor inputs and physical obstacles and tasks can be represented as software objects then a robot may know for example that a collision will occur if it moves left. It may not have to calculate at all as the task and the obstacles are all part of the whole. Once we get there then perhaps a whole new host of problems will face us. We will need to guide and communicate rather than command the machinery.

2.2 MODELS FOR THE DERIVATION OF OUR PROJECT:

2.2.1 Simple automatic media dispenser:

The automatic media dispenser shown in the figure below has been constructed in the Western Regional Research Laboratory from inexpensive parts, enabling an operator to dispense media at many times the speed of handwork.

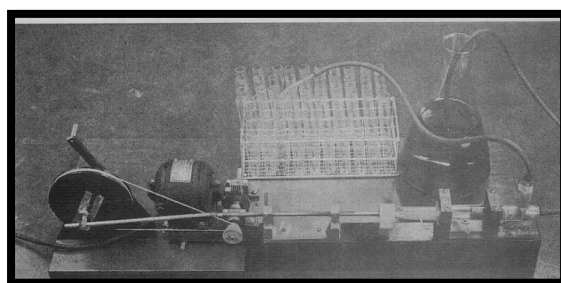


Figure 2.1: Media Dispenser

The essential parts are a 20-ml a syringe with tip end removed, a system of valves and tubes, a driving mechanism and stable mounting. The barrel of the syringe was cut at the aero mark. The open end was connected utilizing a rubber stopper to a valve system, which consists of a glass T and two check valves cut from No. 2 rubber stoppers BS. (Andersen, n.d.)

2.2.2 Validation of a new protocol for ^{18}F -fdg infusion using an automatically combined dispenser and injector system:

2.2.2.1 Purpose:

In nuclear medicine, radiopharmaceuticals are usually administered in unit doses partitioned from multi-dose vials (Boellaard et al., 2010). The partitioning typically takes place in a radiopharmacy, depending on local practice (Merce et al., 2011). Automatic, as opposed to manual, partitioning and administration should reduce radiation exposure of the personnel involved, improve the accuracy of the administration and mitigate contamination (Merce et al., 2011). This study set out to verify and validate the ^{18}F -fluorodeoxyglucose (FDG) administration procedure

performed using Intego™ (MEDRAD, Inc., Warrendale, PA, USA), a combined dispenser and injector system (Guillet et al., 2005). We considered maintenance of sterility and the system's potential to improve, concerning the manual procedure, the accuracy of net administered ^{18}F -FDG radioactivity in patients, and the radiation protection of operators (Covens et al., 2010).

2.2.2.2 Methods:

A media-fill procedure was used to assess whether sterility is maintained during the use of the Intego™ system (Sirna et al., 2010). Simulating a typical working day's setup and use of the system, we investigated the accuracy of the net administered ^{18}F -FDG activity obtained with Intego™ versus the manual dose delivery system (Zanzonico et al., 2008).

We also measured personnel radiation exposure during the use of Intego™ (Demir et al., 2010) and manual administration and recorded and compared environmental doses in the two conditions (Benatar N A et al., 2000).

2.2.2.3 Results:

The radiopharmaceutical remained sterile in all the tests performed. The accuracy of the net ^{18}F -FDG activity delivered to the patients was found to be within 3 % points, as required by European Association of Nuclear Medicine (EANM) guidelines on ^{18}F -FDG imaging procedures. With Intego™, the residual radioactivity in the tubing was 0.20 MBq, corresponding to approximately 0.07 % of the mean activity delivered. With the manual injection, the residual radioactivity in the syringe averaged 7.37 MBq, corresponding to a mean error of 2.9 % in the delivered dose. During the injection step of the positron emission tomography (PET) procedure, whole-body and extremity radiation exposures were significantly reduced with Intego™ by 38 and by 94 %, respectively, compared to the levels associated with manual administration ($p < 0.05$).

2.2.2.4 Conclusion:

Intego™ accurately partitions and administers sterile doses of ^{18}F -FDG from multi-dose vials. Compared with standard manual ^{18}F -FDG administration, the new procedure with an automatic dispensing and injection system greatly reduces the extremity dose to the operator involved in the administration of the radiopharmaceutical (Tandon et al., 2007).

2.2.3 Automatic liquid dispenser:

The dispenser is made from glass tubing and the siphoning action is self-explanatory. The volume of liquid that empties at one time depends on the height C, the width D, the diameter of the S-tube, and also the frequency of emptying. The frequency of emptying is controlled by the constricted tube B which must fit loosely into the S-tube. A pipette tip serves well for this purpose. The frequency of emptying may be further controlled by a Hoffman clamp. The dispensing is intermittent; and the operator can manipulate a rack of tubes, moving the rack over to locate an empty tube under the dispensing arm while the S-tube is filling. Pinch cook A is used when it is desired to stop the dispensing, otherwise, flow through B is continuous. The

apparatus can be used for dispensing serum or melted agar media as well as broth. We have also used it successfully in dispensing Loeffler's medium, and for filling silver nitrate solution into wax ampules for the prevention of ophthalmia neonatorum. With a small amount of practice, one will be able to more than double one's output using the old pinch cock method (Bukoski, n.d.).

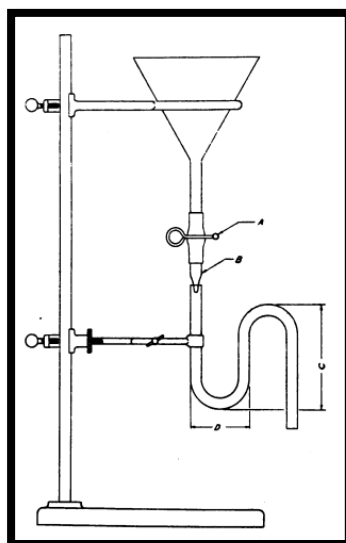


Figure 2.2: Dispenser apparatus

One hundred Loeffler's tubes were filled by each of the two methods in a test run. The time required when using the automatic dispenser was 3 minutes 16 seconds. When using the pinch cock method, 8 minutes and 31 seconds were required. Another advantage of the use of the dispenser is the large saving in media, because only the right amount of medium and no more is dispensed into each tube or flask.

2.3 INPUT DEVICES INCULCATED IN THE MEDIA DISPENSER (STRUCTURE, FUNCTION, AND ITS APPLICATION IN BIOTECHNOLOGY YET NOW) :

2.3.1 Temperature sensor:

The Arduino temperature sensor transforms the ambient temperature into electricity. It also converts the voltage to Celcius, then to Fahrenheit, and displays the Fahrenheit temperature on the LCD panel. We'll utilize a low-voltage temperature sensor (TMP 36). When dealing with huge capacitive loads, such sensors are also stable. It's also acceptable for use in automobiles. TMP 35, TMP 36, and TMP 37 are temperature sensors that have the same properties. The TMP 36 sensor's working voltage spans from 2.7 to 5.5 volts.



Figure 2.3: Temperature sensor

There are three terminals on it, as mentioned below:

DC voltage (pin 1)

On the Arduino UNO board, we'll connect the DC voltage pin to 5V.

Analog voltage output (pin 2)

The Analog voltage output pin will be used as the output.

GND (pin 3)

On the Arduino UNO board, we'll connect the GND pin to Ground.

2.3.2 Limit switches:

A limit switch is an electromechanical device that is activated by an item exerting physical force on it. Limit switches are used to determine whether or not an object is present. Because these switches were initially intended to designate an object's limit of travel, they were given the moniker Limit Switch.

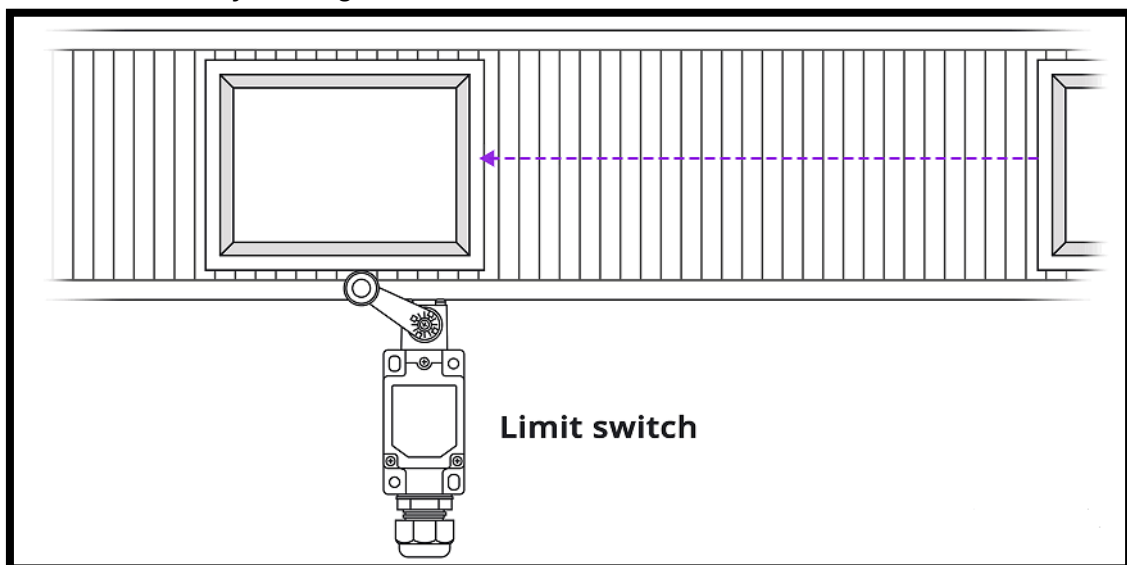


Figure 2.4: Limit switches

2.3.3 Start button:

Start button also called a push-button switch is a small, sealed mechanism that completes an electric circuit when you press on it. When it's on, a small metal spring inside makes contact with two wires, allowing electricity to flow. When it's off, the spring retracts, contact is interrupted, and the current won't flow.

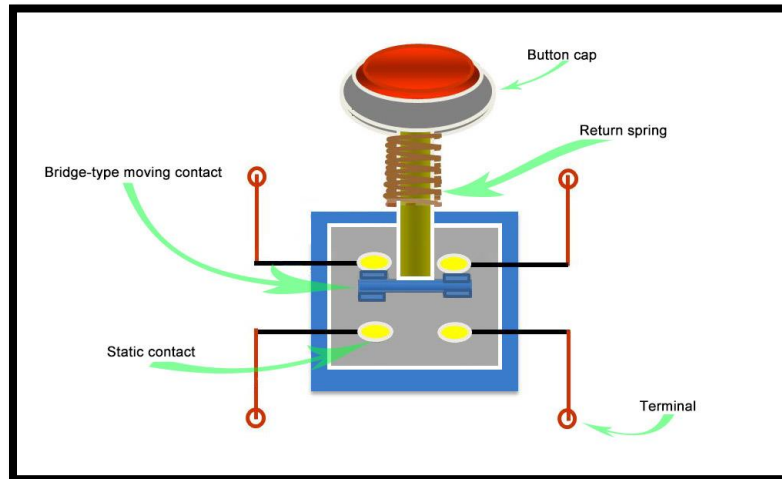


Figure 2.5: Start Button

2.3.4 Weighing scale (cantilever beam):

A Cantilever beam is one of the widely used mechanical devices used for force measurement. It converts applied force into mechanical displacement. By measuring the displacement, we get a measure of the applied force.



Figure 2.6: Weighing scale (cantilever beam):

2.4 CONTROLLER DEVICES USED IN THE MEDIA DISPENSER:

2.4.1 Arduino mega:

The Mega 2560 is an ATmega2560-based microcontroller board. It contains 54 digital input/output pins, 16 analog inputs, 4 UARTs (hardware serial ports), a 16 MHz crystal oscillator, a USB connection, a power connector, an ICSP header, and a reset button. It comes with everything you'll need to get started with the microcontroller; simply plug it into a computer with a USB connection or power it with an AC-to-DC converter or battery. Most shields created for the Uno and previous boards Duemilanove or Diecimila is compatible with the Mega 2560 board. The Arduino Mega 2560 is a successor to the Arduino Mega.

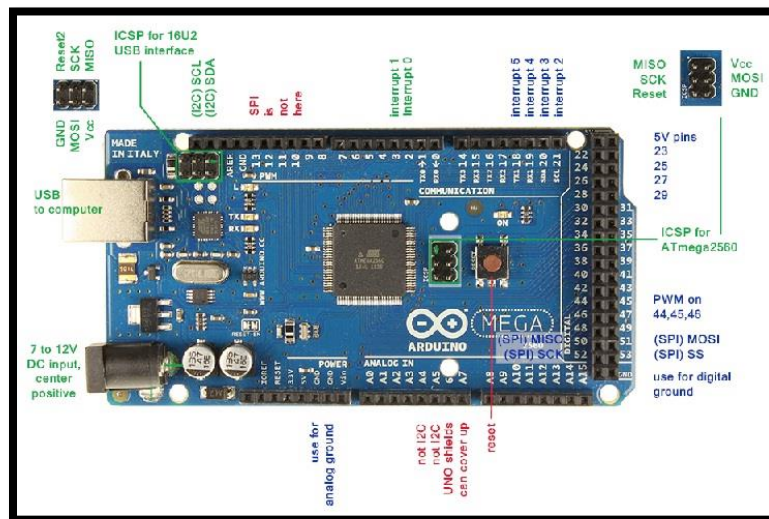


Figure 2.7: Arduino mega

2.4.2 Motor drivers:

Motor drivers serve as a connection between motors and control circuits. The controller circuit operates on low current signals, but the motor requires a large amount of current. The purpose of motor drivers is to convert a low-current control signal into a higher-current signal capable of driving a motor.

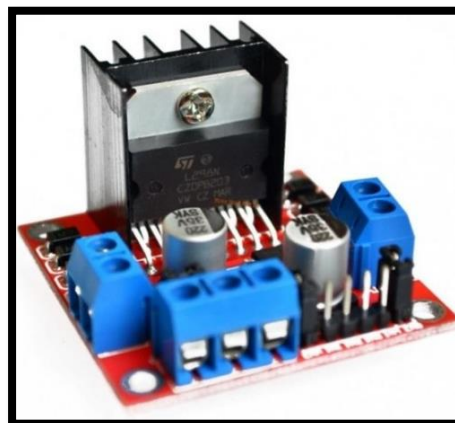


Figure 2.8: Motor drivers

2.4.3 Relay board:

Computer boards featuring a variety of relays and switches are known as relay boards. They are meant to manage the voltage supply and feature input and output terminals. For each of the onboard relay channels, relay boards enable separately programmable, real-time control.

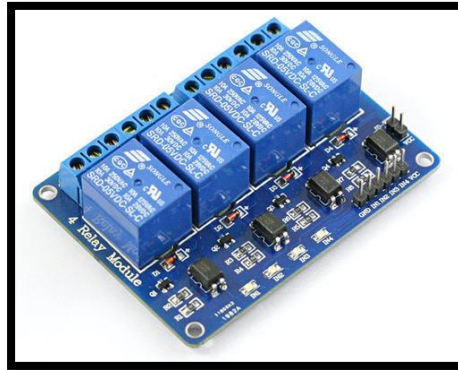


Figure 2.9: Relay board

2.5 OUTPUT DEVICES:

2.5.1 Servo motor:

A servomotor is a linear or rotary actuator that provides for exact control of position, acceleration, and velocity in linear or angular directions. It is made comprised of a motor and a position feedback sensor. It also necessitates a complex controller, which is frequently a separate module created exclusively for servomotors. A servo motor is typically a basic DC motor with added servomechanism to regulate specified angular rotation (a typical closed-loop feedback control system). Servo systems are frequently employed in industrial applications nowadays.



Figure 2.10: Servo motor

2.5.2 Stepper motor:

A stepper motor is a mechanical device that transforms electrical energy into mechanical energy. It's also a brushless, synchronous electric motor with the ability to partition a whole rotation into a large number of stages. As long as the motor is properly sized for the application, the position of the motor may be precisely regulated without the need for any feedback device. Switched reluctance motors are comparable to stepper motors. When a pulse of electricity is applied to the stepper motor, the theory of operation for magnets is used to cause the motor shaft to turn a specific distance. There are eight poles on the stator and six poles on the rotor. To complete one complete rotation, the rotor will require 24 electrical pulses to move the 24 steps. Another way to put it is that for each pulse of power received by the motor, the rotor will rotate precisely 15° .



Figure 2.11: Stepper motor

2.5.3 DC motor:

A DC motor, also known as a direct current motor, is an electrical machine that uses direct current to convert electrical energy into mechanical energy. A magnetic field is formed in the stator of a DC motor when it is turned on. The field causes the rotor to revolve by attracting and repelling magnets on the rotor. The commutator, which is hooked to brushes connected to the power source, supplies current to the motor's wire windings to keep the rotor revolving. One of the advantages of DC motors over other types of motors is their ability to precisely adjust speed, which is essential for industrial machines. DC motors can start, stop, and reverse quickly, which is critical for managing the operation of manufacturing equipment.



Figure 2.11: DC motor

2.5.4 Heating element:

A heating element is a substance or device that uses the Joule heating principle to transform electrical energy into heat or thermal energy. The phenomenon of joule heating occurs when a conductor develops heat as a result of the flow of electric current. As the electric current passes through the material, electrons or other charge carriers hit the conductor's ions or atoms, causing atomic-scale friction. Heat is produced as a result of friction. The quantity of heat created by the passage of electricity in a conductor is described by Joule's first law (Joule-Lenz law). Except for a unique type of material known as superconductors, Joule heating is seen in all conducting materials at variable intensities. Electrically conductive materials create less heat because charge carriers can readily pass through them, whereas materials with high electric resistance generate more heat. Superconductors, on the other hand, enable electricity to flow but do not generate heat. The heat from conductors is usually considered an energy loss. The use of electrical energy to power powered equipment causes needless heating in the form of copper loss, which does not provide any beneficial work.

In a sense, electrical heating components are nearly 100 percent efficient since all given energy is transformed into the desired form. Heating elements can transmit energy not just through heat but also through light and radiation. This is only true for perfect resistors, though. The intrinsic capacitance and inductance of the material, which transforms electrical energy into electric and magnetic fields, might result in small losses. When considering the entire heater system, losses come from the process fluid or the heater itself dissipating heat into the external environment. As a result, the system must be separated to fully use the heat created.



Figure 2.12: Heating element

2.5.5 Water pump:

DC pumps transfer fluid in several ways by using direct current from a motor, battery, or solar power. Typical DC voltages for motorized pumps are 6, 12, 24, or 32 volts. Photovoltaic (PV) panels with solar cells that create direct currents when exposed to sunlight are used in solar-powered DC pumps. DC (direct current) pumps have the benefit of being able to work straight from a battery, making them more handy and portable than AC (alternating current) pumps. Because AC systems often require a controller to govern speed, they are easy to handle and control. DC pumps are also more energy-efficient. AC pumps, on the other hand, are often intended for faster speeds and longer bursts of power. In addition, they have a longer operational life than DC pumps.



Figure 2.13: Water pump:

2.5.6 UV light:

UV is a kind of electromagnetic radiation with wavelengths ranging from 10 nm (corresponding frequency: 30 PHz) to 400 nm (750 THz), which is shorter than visible light but longer than X-rays. UV radiation is found in sunshine and accounts for around 10% of the Sun's total electromagnetic radiation output. Electric arcs and specialty lights, such as mercury-vapor lamps, tanning lamps, and black lights, can also create it. Although long-wavelength ultraviolet cannot ionize atoms because its photons lack the energy to do so, it may initiate chemical processes and make many things light or fluoresce. As a result, UV's chemical and biological impacts are more than its simple heating effects, and UV radiation has a wide range of practical uses. As a result, UV's chemical and biological impacts outweigh its basic heating effects, and many of UV's practical applications stem from its interactions with organic molecules.

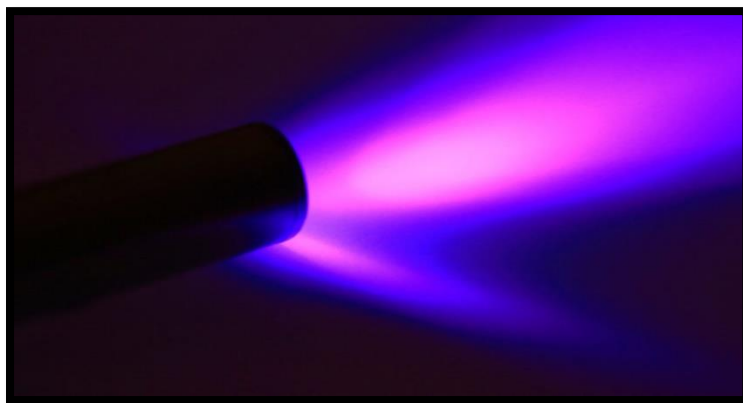


Figure 2.14: UV light:

3. AIM AND SCOPE:

The aim of this machine is to:

- To **increase productivity, operator safety, and media quality**.
- To **save time** by automating one of the tedious and most essential prerequisite for all research in microbiology and cell culture.
- To **minimize human error** while measuring amount of different components of media.
- To avoid contamination due to manual preparation .

The scope of this machine is that it can be used in various biological research areas like Microbiology, Molecular biology and related fields where the preparation of media becomes the primary aspect of completing the project.

4. MATERIALS AND EXPERIMENTAL METHOD:

4.1 MATERIALS REQUIRED:

- 4.1.1 Temperature sensor
- 4.1.2 Limiting switch
- 4.1.3 Start button
- 4.1.4 Kill switch
- 4.1.5 Weighing Scale (cantilever beam)
- 4.1.6 Arduino mega
- 4.1.7 Motor drivers
- 4.1.8 Relay Board
- 4.1.9 Servo motor
- 4.1.10 Stepper motor
- 4.1.11 DC motor
- 4.1.12 AC to DC converter
- 4.1.13 Heating element
- 4.1.14 Water pump
- 4.1.15 UV light
- 4.1.16 Peptone
- 4.1.17 Dextrose
- 4.1.18 Agar
- 4.1.19 Meat extract
- 4.1.20 Potato extract
- 4.1.21 NaCl
- 4.1.22 Distilled water
- 4.1.23 Petri dishes
- 4.1.24 Conical flask

4.2 METHODOLOGY:

4.2.1 Construction of the SPM:

4.2.1.1 Formulation of the SPM:

The idea of an automated machine for media preparation was formulated. The components required for the machine were designed. All the circuits were constructed and tested by proteus and manually.

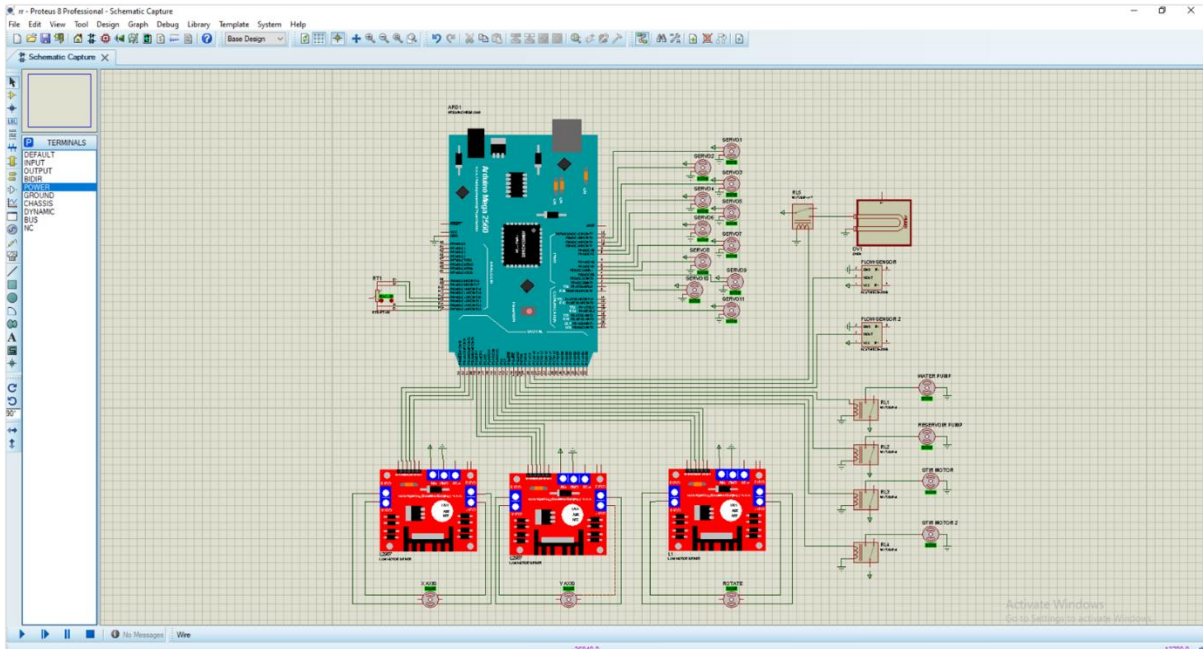


Figure 4.1: Circuit simulation using proteus

4.2.1.2 Programming:

The embedded C program were written and verified using arduino.ide compiler and simulated.

The coding of embedded C program:

```
#include <Servo.h>
Servo myservo1;
Servo myservo2;
Servo myservo3;
Servo myservo4;
Servo myservo5;
Servo myservo6;
Servo myservo7;//powder platform
Servo myservo8;//tool changer
Servo myservo9;
Servo myservo10;
Servo myservo11;
```

```

#include <Stepper.h>
#include <OneWire.h>
#include <DallasTemperature.h>
#define ONE_WIRE_BUS 31

OneWire oneWire(ONE_WIRE_BUS);
DallasTemperature sensors(&oneWire);

#define button1 22
#define PumpPin 32
#define StirPin 33
#define HeatingPin 34
#define pump2pin 35
#define watervalue 36
#define medvalve 37

int buttonstate = 0;

const int stepsPerRevolution = 200;

Stepper myStepper1(stepsPerRevolution, 23, 24, 25, 26);

Stepper myStepper2(stepsPerRevolution, 27, 28, 29, 30);

Stepper myStepper3(stepsPerRevolution, 40, 41, 42, 43);

const int FlowPin = 38;

volatile int Pulse_Count;
unsigned int Liter_per_hour;
unsigned long Current_Time, Loop_Time;

const int Flow2Pin = 39;

volatile int Pulse_Count2;
unsigned int Liter_per_hour2;
unsigned long Current_Time2, Loop_Time2;

void setup()
{

```

```

myservo1.attach(2);
myservo2.attach(3);
myservo3.attach(4);
myservo4.attach(5);
myservo5.attach(6);
myservo6.attach(7);
myservo7.attach(8);
myservo8.attach(9);
myservo9.attach(10);
myservo10.attach(11);
myservo11.attach(12);

myStepper1.setSpeed(60);
myStepper2.setSpeed(60);
myStepper3.setSpeed(60);

sensors.begin();
Serial.begin(9600);

PinMode (FlowPin, INPUT);
pinMode (Flow2Pin, INPUT);
pinMode (button1, INPUT);
pinMode (PumpPin, OUTPUT);
pinMode (HeatingPin, OUTPUT);
pinMode (StirPin, OUTPUT);

attachInterrupt(0, Detect_Rising_Edge, RISING);
sei();
Current_Time = millis();
Loop_Time = Current_Time;

attachInterrupt(0, Detect_Rising_Edge2, RISING);
sei();
Current_Time2 = millis();
Loop_Time2 = Current_Time2;
}

void loop() {
  myservo1.write(0);
  myservo2.write(0);
  myservo3.write(0);
  myservo4.write(0);
  myservo5.write(0);
  myservo6.write(0);

```

```

myservo7.write(0);
myservo8.write(0);
myservo9.write(0);
myservo10.write(0);
myservo11.write(0);

if (button1 == HIGH)
{

    myStepper1.step(33);
    delay(500);
    Serial.print("Lid is positioned to filling area 1");
    myservo1.write(90);
    delay(1000);
    myservo1.write(0);
    delay(1000);
    Serial.print("Solution1 is filled");

    myStepper1.step(33);
    delay(500);
    Serial.print("Lid is positioned to filling area 2");
    myservo2.write(90);
    delay(1000);
    myservo2.write(0);
    delay(1000);
    Serial.print("Solution2 is filled");

    myStepper1.step(33);
    delay(500);
    Serial.print("Lid is positioned to filling area 3");
    myservo3.write(90);
    delay(1000);
    myservo3.write(0);
    delay(1000);
    Serial.print("Solution3 is filled");

    myStepper1.step(33);
    delay(500);
    Serial.print("Lid is positioned to filling area 4");
    myservo4.write(90);
    delay(1000);
    myservo4.write(0);
    delay(1000);
    Serial.print("Solution4 is filled");
}

```

```

myStepper1.step(33);
delay(500);
Serial.print("Lid is positioned to filling area 5");
myservo5.write(90);
delay(1000);
myservo5.write(0);
delay(1000);
Serial.print("Solution5 is filled");

```

```

myStepper1.step(33);
delay(500);
Serial.print("Lid is positioned to filling area 6");
myservo6.write(90);
delay(1000);
myservo6.write(0);
delay(1000);
Serial.print("Solution6 is filled");

```

```

myStepper1.step(-198);
delay(500);
Serial.print("Solution is ready to be filled in concial container");

```

```

myservo7.write(180);
delay(5000);
myservo7.write(0);
delay(500);
Serial.print("Solution is filled in the conical container");

```

```

////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////

```

```

myservo8.write(0); //choose the tool
delay(500);

```

```

myStepper2.step(100); //toolpost dipped into the flask
delay(500);

```

```

//flow sensor
Current_Time = millis();
if(Current_Time >= (Loop_Time + 1000))
{
    Loop_Time = Current_Time;
    Liter_per_hour = (Pulse_Count * 60 / 7.5);
    Pulse_Count = 0;
}

```

```

    Serial.print(Pulse_Count);
}
if(Pulse_Count <1000) //no of pulses to turn off the valve
{
    digitalWrite(watervalue, HIGH); //turn on the water valve till the desired volume
is reached

}
else
{
    digitalWrite(watervalue, LOW);
}

digitalWrite(StirPin,HIGH);
sensors.requestTemperatures();
Serial.print("Celsiustemperature");
Serial.print(sensors.getTempCByIndex(0));
if (sensors.getTempCByIndex(0) > 100) // if less than 100c turn on heater
{
    digitalWrite(HeatingPin, LOW);
}
else
{
    digitalWrite(HeatingPin, HIGH);
}

digitalWrite(StirPin,LOW);

myStepper2.step(-100);
delay(500);

myservo8.write(180); //turn the tool from mix setup to drain setup
delay(500);

myStepper2.step(100);
delay(500);

digitalWrite(PumpPin,HIGH);
delay(50000); //time to drain the flask and fill the reservoir

digitalWrite(PumpPin,LOW);
delay(500);

myStepper2.step(-100);

```

```

delay(500);

myservo8.write(0); //turn the tool from drain setup to mix setup
delay(500);

////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////

myservo9.write(180); //push the dish
delay(500);

myservo9.write(0);
delay(500);

//flow sensor 2
Current_Time = millis();
if(Current_Time2 >= (Loop_Time2 + 1000))
{
  Loop_Time2 = Current_Time2;
  Liter_per_hour2 = (Pulse_Count2 * 60 / 7.5);
  Pulse_Count2 = 0;
  Serial.print(Pulse_Count2);
}
if(Pulse_Count2 <50) //no of pulses to turn off the pump
{
  digitalWrite(medvalve, HIGH); //turn on the medium valve till the desired volume
is reached

}
else
{
  digitalWrite(medvalve, LOW);
}

myservo10.write(180); //push the dish top
delay(500);

myservo10.write(0);
delay(90000); // cool time (1000ms = 1sec)

myservo11.write(180); //push the dish top
delay(500);

myservo11.write(0);
delay(2000);

```

```

myStepper1.step(50); // steps to turn 180degree
delay(2000);

myStepper1.step(-50);
delay(500);
Serial.print("petridish is out");
}

}

void Detect_Rising_Edge ()
{
    Pulse_Count++;
}

void Detect_Rising_Edge2 ()
{
    Pulse_Count2++;
}

```

4.2.1.3 Assembly and Fabrication:

The frame, guide rail and housing for stepper mounted components were fabricated individually and were assembled. The electronic components and actuators were mounted according to the circuit diagram designed. Harness routing were wired. Pipelines for water supply and drainage inside the SPM were arranged.

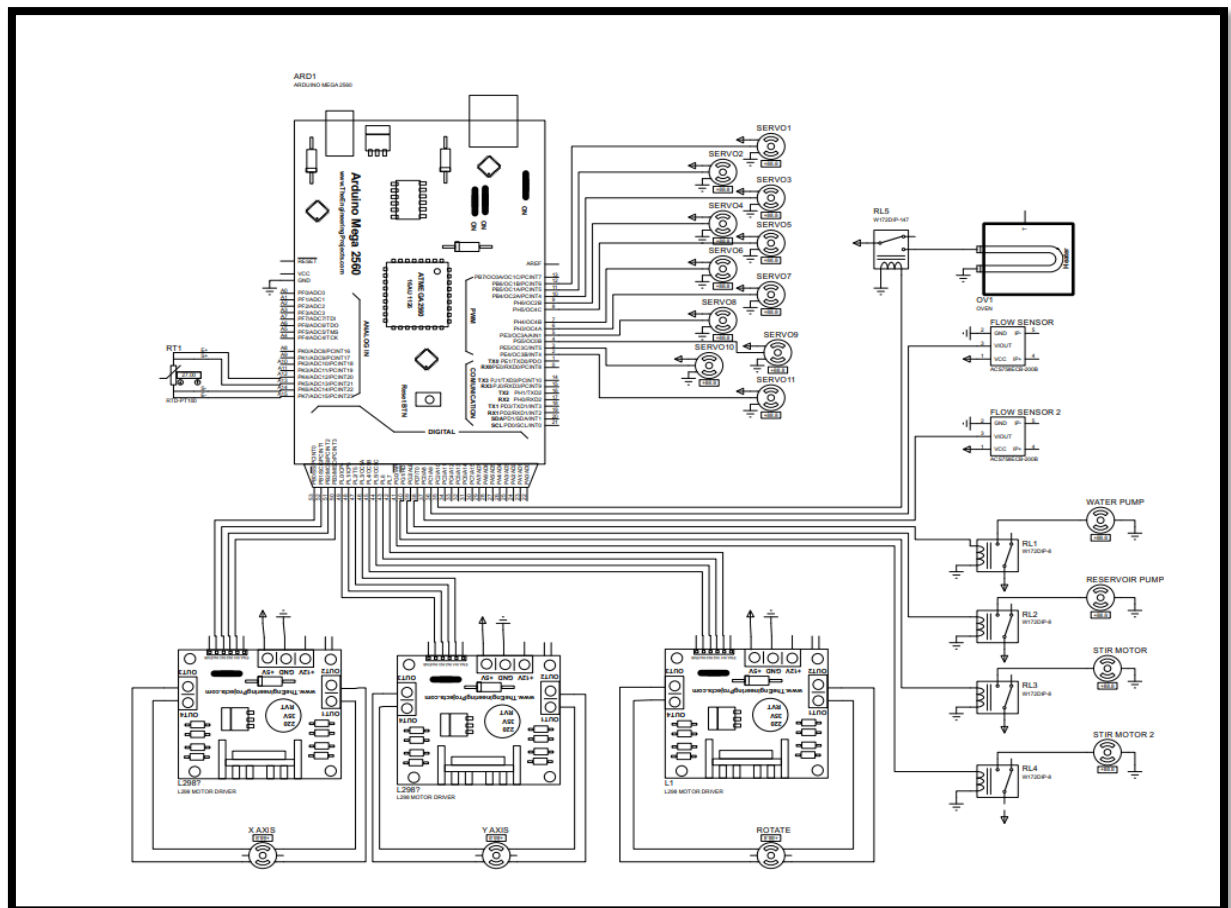


Figure 4.2: Circuit diagram

4.2.2 Working of SPM:

4.2.1.1 Input of media composition:

The composition of media required were coded. SPM was coded to prepare two types of media prepared. The types include Nutrient Agar and Potato Dextrose Agar. The composition of NA (Nutrient Agar) (100 ml):

- Peptone - 1g
- Meat extract B # - 1g
- Sodium chloride - 0.5 g
- Agar - 1.2 g
- Sterilized mono distilled water – 100ml

The composition of PDA (Potato Dextrose Agar) 100 ml:

- Potato infusion form – 20 g
- Dextrose – 1 g
- Agar – 1g
- Sterilized mono distilled water – 100ml

4.2.1.2 Loading of glass wares:

The glassware required for storing and collecting media are sterilized and stacked.

4.2.1.3 Sterilization:

The components and vessels used are sterilized using UV LED light (Muramoto et al., 2014). UV light helps to eradicate all the microorganisms by denaturing nucleic acids. Especially UV even sterilizes virus on the surface. This maintains the machine in a sterile condition.

4.2.1.4 Weighing and mixing of components:

The different components are weighed by the weighing scale (cantilever beam) (Hambir et al., 2019) mounted on the stepper motor (Jirasuwankul & Manop, 2017), which moves and collects the compounds and drops into the conical flask. Distilled water is added using a valve and flow sensor. A stack of stirrer, temperature sensor (Ukil et al., 2012), heating element and pump (Hepbasli & Kalinci, 2009) are inserted into the conical flask. In the prepared media, the optimum conditions are checked.

4.2.1.5 Storing and Collection of Prepared Media:

Once all the conditions are satisfied the prepared media is transferred into a reservoir. The stacked petri dishes are moved using servo motor-controlled path (Haidar et al., 2013). The required amount of culture media is poured in petri dish using a flow sensor. The culture media in Petri dish are dispatched out from SPM for further usage.

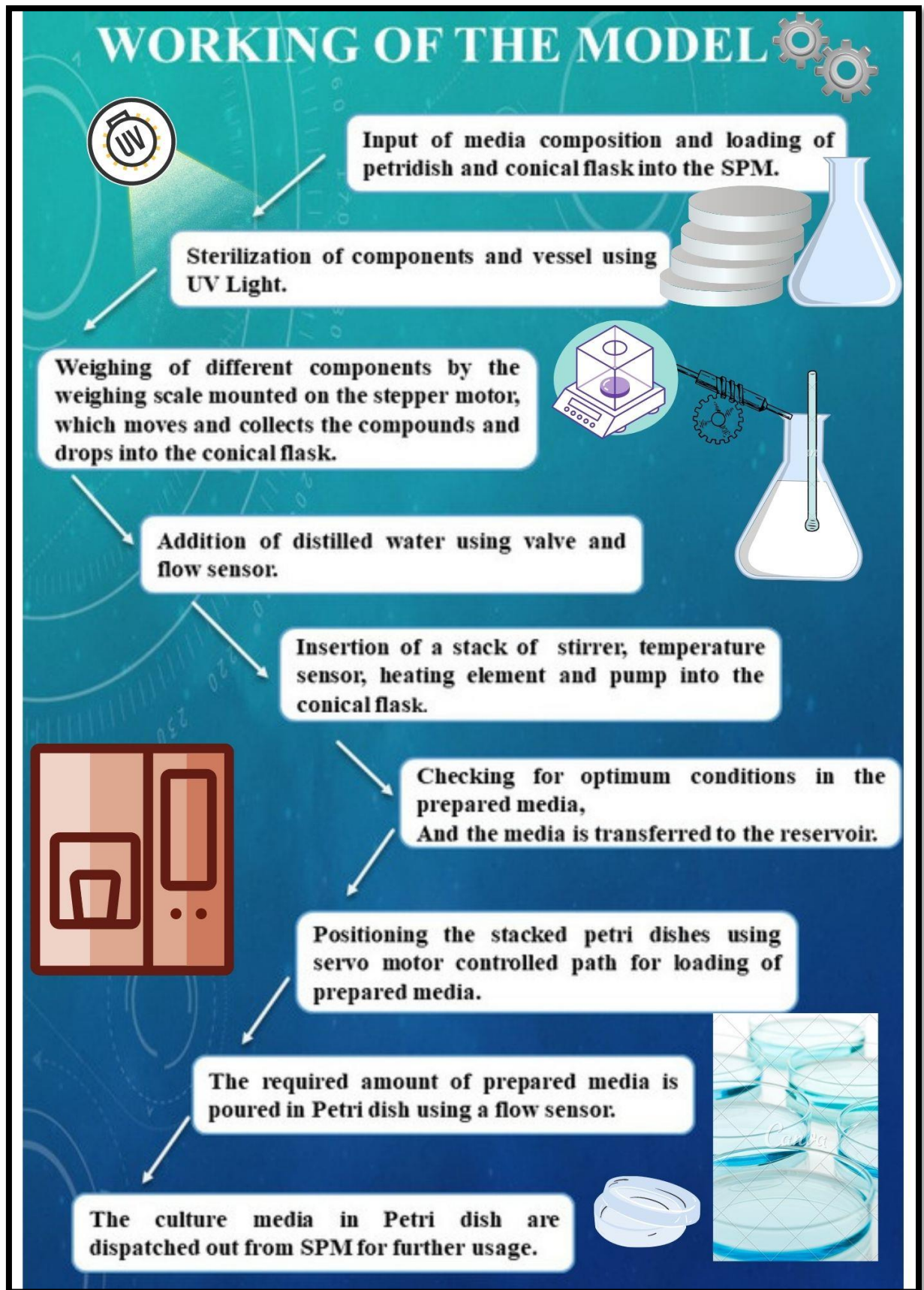


Figure 4.3: Working of the model

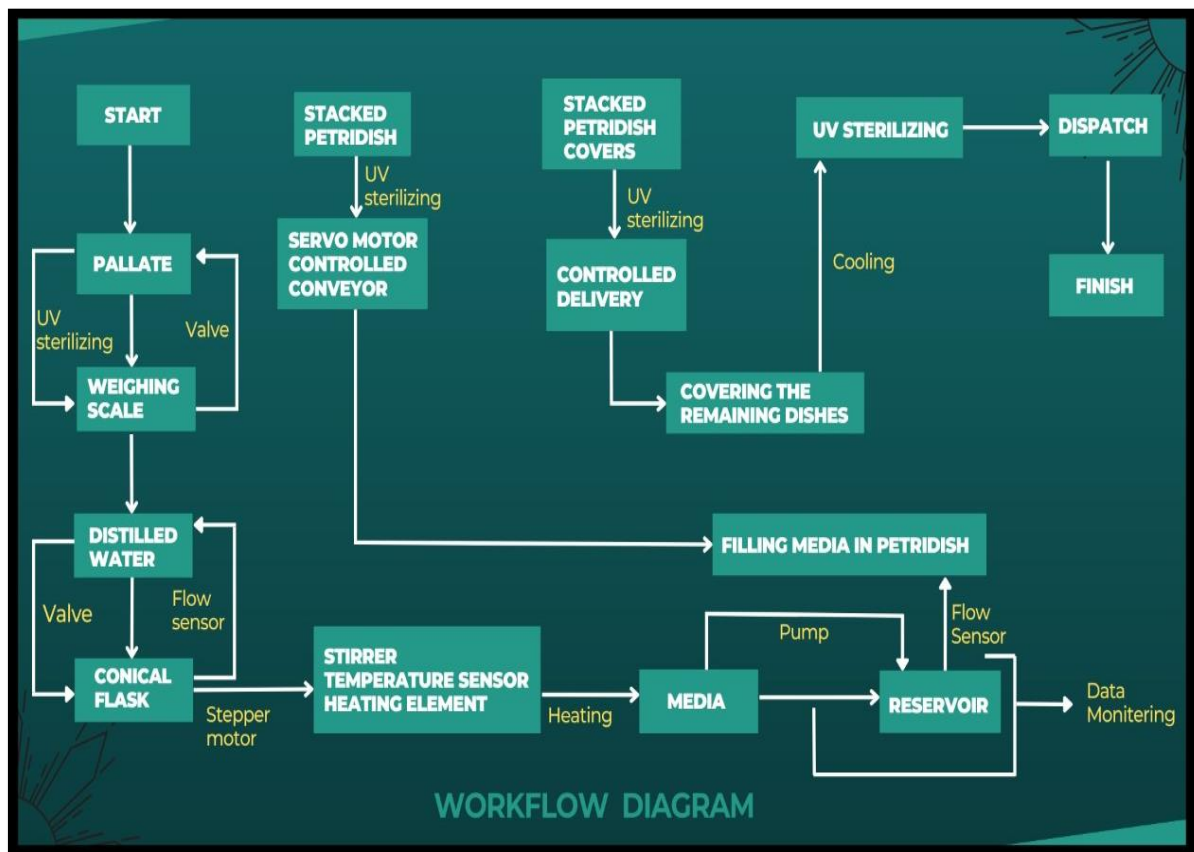


Figure 4.4 : Work flow chart

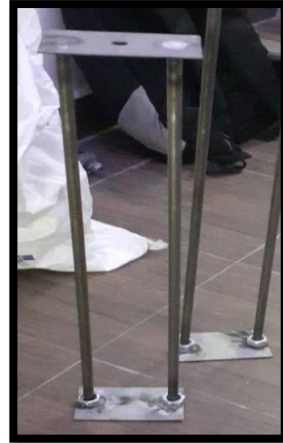
5. RESULTS AND DISCUSSION:

The implementation of an automated media preparing and dispensing system reduced overall time consumption in sterilization, preparation and distributing it into different vessel. Furthermore, no contamination was seen when the media was prepared. Depending on the sample or assay automated media preparing and dispensing system will fill agar, broths or any other liquid into Petri plates.

By using automated media preparing and dispensing system the entire media preparation process is standardized and under control from start to finish. The media is stirred homogenously using the stirrer which ensures the uniform distribution of the components. Agar dispensing at optimal temperature guarantees quick solidification and reduced condensation to limit contamination risk. This system also enhances safety for laboratory personnel - no hot agar/broths to carry around. With the ever-increasing demand to do more with less, these automated solutions to media preparation and dispensing are becoming a necessity for the modern successful microbiology testing laboratory.

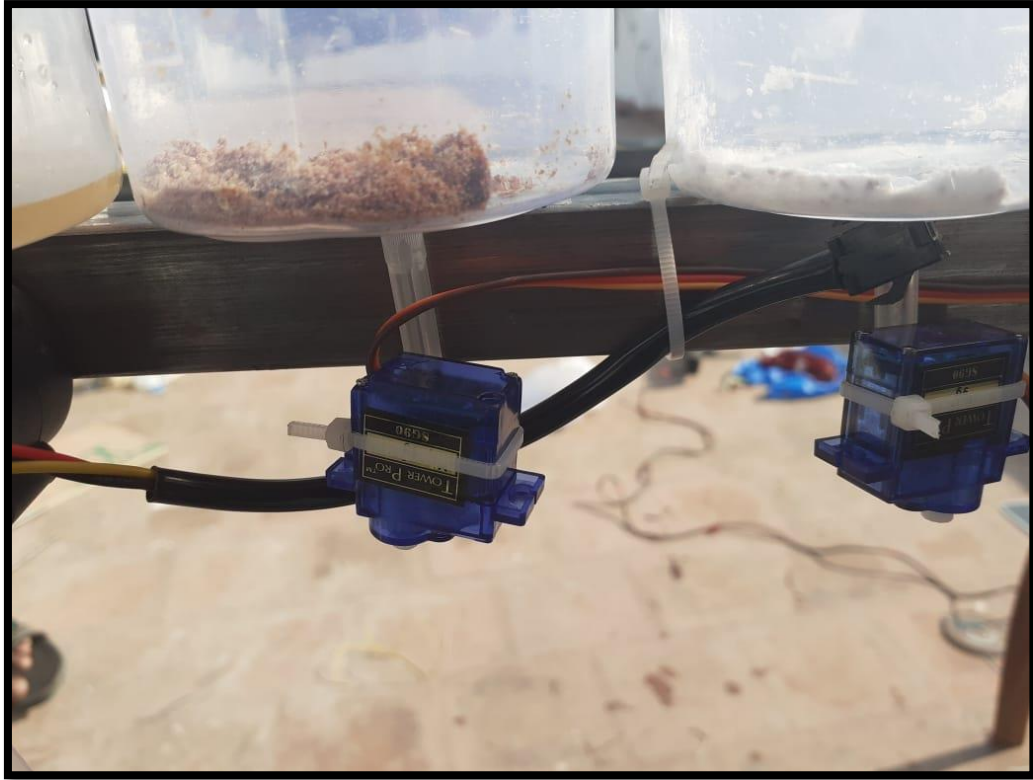
Ultimately the choice of whether to use an automated or semi-automated system for media preparation and dispenser depends on cost effectiveness of the system. Market demands and what the market will pay for automated media preparing and dispensing system is an important consideration.

MAKING OF THE MACHINE:



AUTOMATIC MEDIA PREPARING MACHINE :





6. SUMMARY AND CONCLUSION:

6.1 SUMMARY:

The automatic media dispenser that we have developed is a novel machine that would help preparing media in bulk and also it helps in maintaining the accuracy. The idea of developing this media dispenser especially for the microbiology field is to help the scientists, the technical assistants and the PHD students who are working long hours on developing their theoretical ideas into the practical applicability. But during this tedious process they spend ample amount of time in preparing media for culturing their desired micro-organism.

Also, in central research institutes like ICAR (The Indian Council of Agricultural Research) and ICMR (The Indian Council of Medical Research), the bulk preparation of media is more often necessary for various purposes. And this microbiological aspect of media preparation is the basis for growing micro-organisms which is the key sample for many projects in different departments like Molecular biology, Genetic engineering, Bio engineering and many others.

The automatic media dispenser has various advantages over manual method of preparing media. The key advantage is that we use UV (Ultra Violet) light radiation with autoclaving mechanism by using heating element to heat the prepared media to about 121°C for sterilizing our media which helps us to assure that the during the process of media preparation the contamination can be reduced to its maximum. Also, as the weighing of the components is done by the machine in which we have already set the code and did analysis, the precision of weighing different components will be greater than what we have been doing manually until this time, this helps us in finding the adaptive media for our desired micro-organism in much easier way. To make it user-friendly we have devised our machine in such a way that even school going child can handle it, this opens the door for junior scientists who are going to be the future game changers, for this we have already set the modes in which the data for specific components with the volume required.

The added advantage of this media dispenser is that we have coded the machine and implemented computer vision by making it accurate to fill the media in the Petri-plate as soon as the temperature comes down to room temperature, for this we use temperature sensor.

6.2 CONCLUSION:

The future implementations over the machine we have prepared would be:

- ◆ Implementing microprocessor instead of microcontroller
- ◆ IOT can be enabled for hassle free operation and convenience
- ◆ Autoclave can be added to reduce contamination rate to near zero
- ◆ Batch printing and packing can be implemented to have good traceability

Thus, the automatic media dispenser that is being developed is far more better than the classical manual method of preparing media as it works on the disadvantages of the latter and gives good results.

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APPENDIX

- Potato dextrose agar (100ml):

Potatoes infusion form – 20g

Dextrose – 1gm

Agar – 1gm

Sterilized mono distilled water – 100ml

- Nutrient Agar (100 ml):

Peptone - 1g

Meat extract B # - 1g

Sodium chloride - 0.5 g

Agar - 1.2 g

Sterilized mono distilled water – 100ml



INTERDISCIPLINARY PROJECT

DEVELOPMENT AND FABRICATION OF AUTOMATED MEDIA PREPARING MACHINE

BY:

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DEPARTMENT OF MECHATRONICS

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EXTERNAL GUIDE: *Dr LILLY MERCY J*

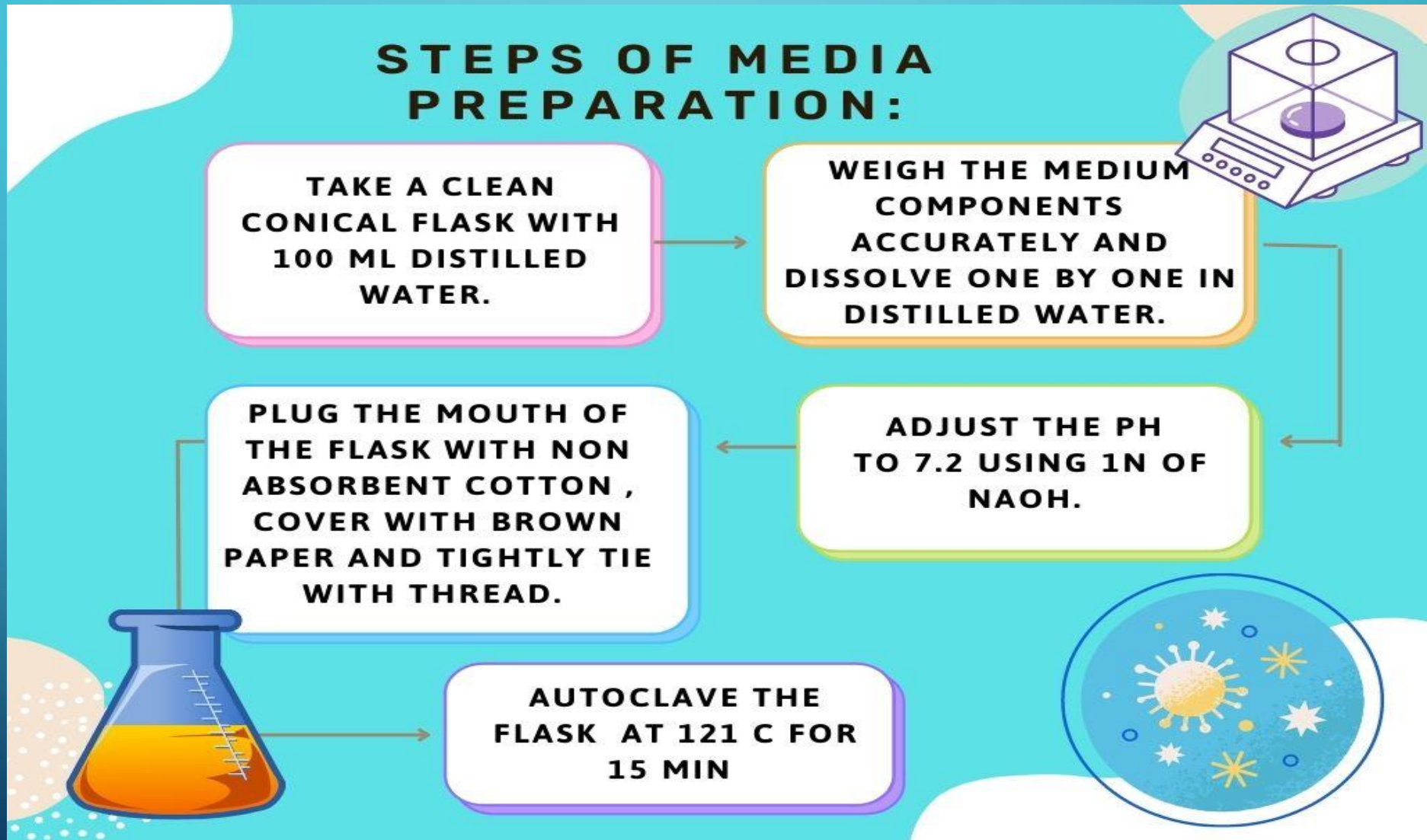
ABSTRACT :

- Microorganisms are almost widespread, numerous, and indispensable for human life. One of the prerequisites for studying them is the preparation of standard culture media. [1-2]
- The production and delivery of culture medium are one of the most time-consuming and tedious operations in the modern microbiology laboratory and poses a safety risk to the personnel who prepare the media (transferring heavy, hot flasks from the autoclave to the benchtop). [3-6]
- There are now reliable solutions to these process challenges in the form of high capacity automated media sterilizers and plate pourers. [7]
- We built a fully automated unit that prepares culture media formulations in independent culture in a sterilized environment. The media preparation system will automatically mix, sterilize and automatically dispense the proper volume into the sample container quickly and accurately. [8-12]

OBJECTIVES :

- To **increase productivity, operator safety, and media quality.**
- To **save time** by automating one of the tedious and most essential prerequisite for all research in microbiology and cell culture.
- To **minimize human error** while measuring amount of different components of media.
- To avoid contamination due to manual preparation .

CLASSICAL PREPARATION OF MEDIA :



COMPOSITION OF MEDIA :

- Potato dextrose agar (100ml): [20]
 - Potatoes infusion form – 20g
 - Dextrose – 1gm
 - Agar – 1gm
 - Sterilized mono distilled water – 100ml



- Nutrient Agar (100 ml): [19]

- Peptone - 1g
 - Meat extract B # - 1g
 - Sodium chloride - 0.5 g
 - Agar - 1.2 g
 - Sterilized mono distilled water – 100ml



ELECTRONIC COMPONENTS :

- INPUT DEVICES

- TEMPERATURE SENSOR
- LIMIT SWITCHES
- START BUTTON
- WEIGHING SCALE (cantilever beam)

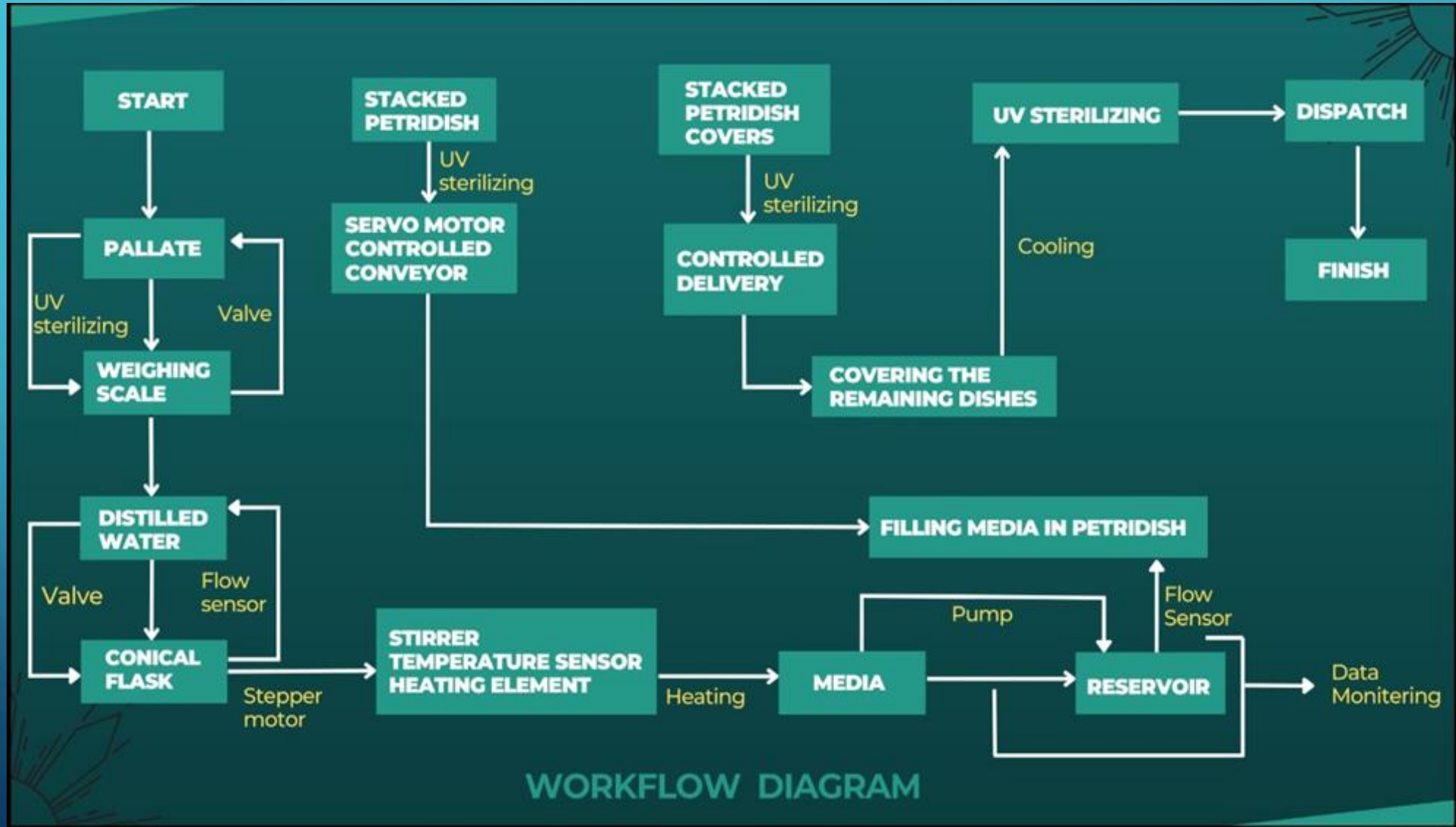
- CONTROLLERS

- ARDIUNO MEGA
- MOTOR DRIVERS
- RELAY BOARD

- ACTUATORS (output)

- SERVO MOTOR
 - STEPPER MOTOR
 - DC MOTOR
 - HEATING ELEMENT
 - WATER PUMP
- -UV LIGHT

METHODOLOGY :



WORKING OF THE MODEL



Input of media composition and loading of petridish and conical flask into the SPM.

Sterilization of components and vessel using UV Light.



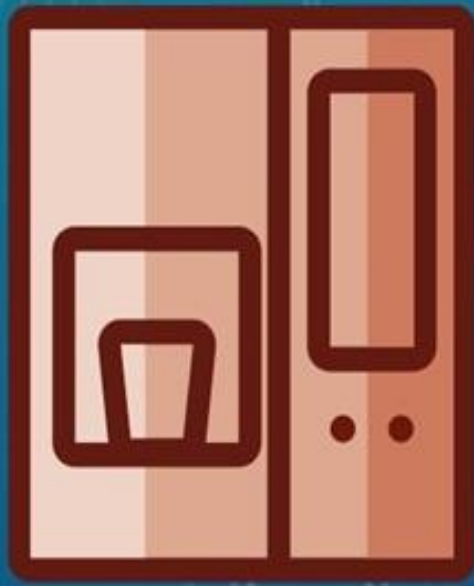
Weighing of different components by the weighing scale mounted on the stepper motor, which moves and collects the compounds and drops into the conical flask.



Addition of distilled water using valve and flow sensor.



Insertion of a stack of stirrer, temperature sensor, heating element and pump into the conical flask.



Checking for optimum conditions in the prepared media,
And the media is transferred to the reservoir.

Positioning the stacked petri dishes using
servo motor controlled path for loading of
prepared media.

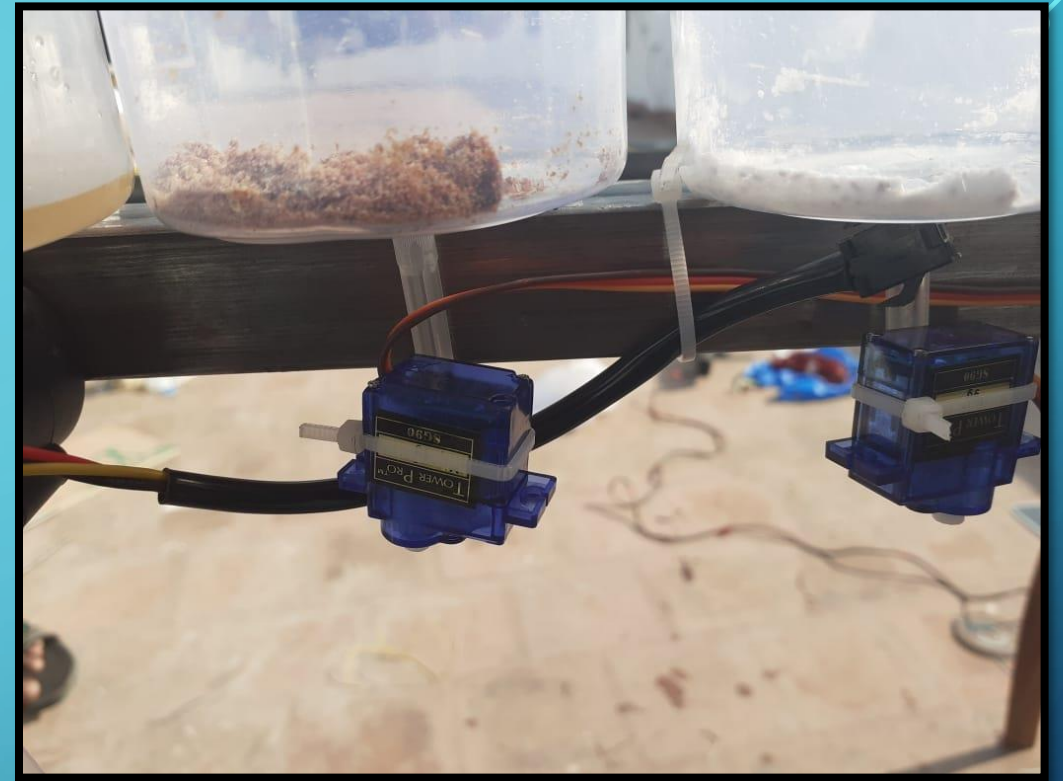
The required amount of prepared media is
poured in Petri dish using a flow sensor.

The culture media in Petri dish are
dispatched out from SPM for further usage.



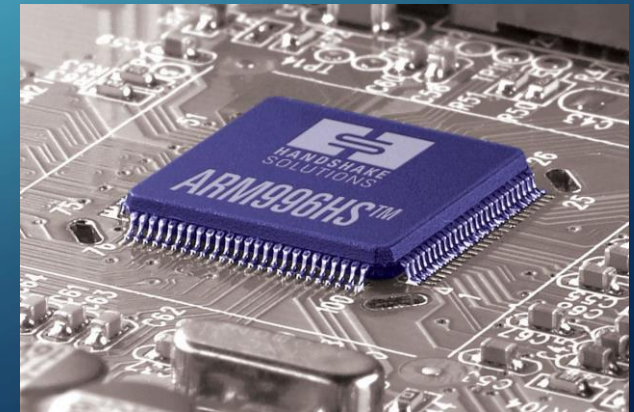
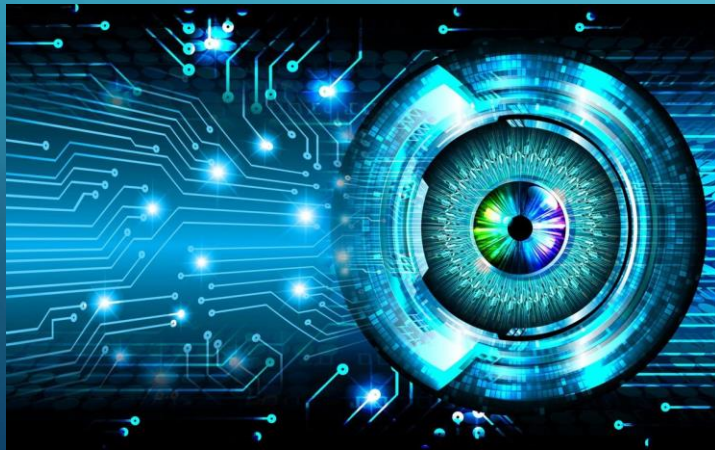
COMPLETED MACHINE:





FUTURE SCOPE :

- Computer vision can be used to improve accuracy
- A microprocessor can be used instead of microcontroller
- IOT can be enabled for hassle free operation and convenience
- Autoclave can be added to reduce contamination rate to near zero
- Batch printing and packing can be implemented to have good traceability.

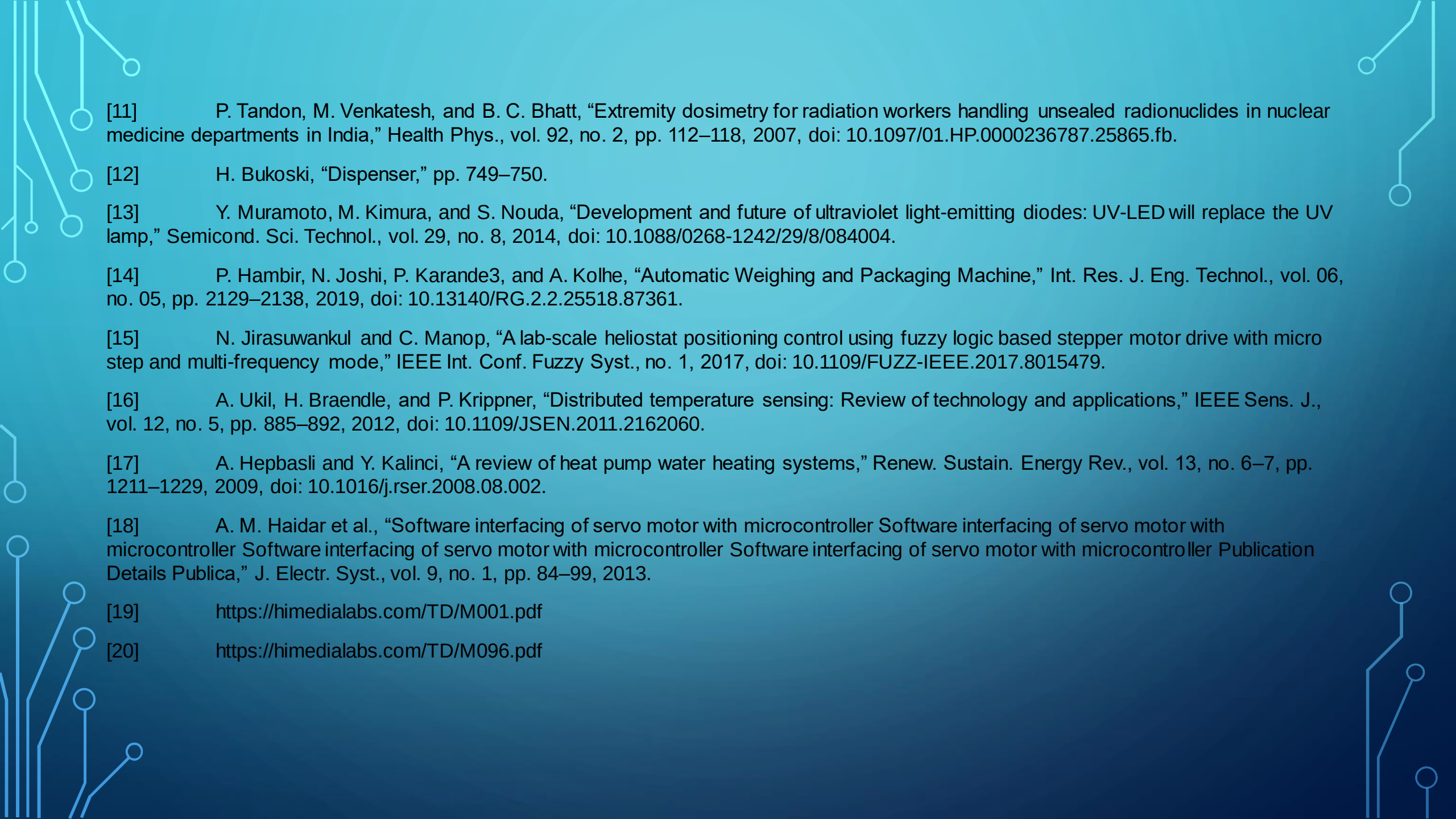


CONCLUSION :

- Laboratories that use this automated technology will see considerable improvements in media quality and production efficiency, as well as significant labour savings.
- These instruments aid in increasing productivity, operator safety, and media quality.
- These automated media preparation and dispensing systems are becoming a need for the modern effective microbiological testing laboratory, as the demand to do more with fewer increases.

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