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Enzyme-Linked Immunosorbent Assay using 96-well plate

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ABSTRACT: Colorimetry is the technique that is frequently used in biochemical investigations. This involves the quantitative estimation of colours. This means that if you want to measure the quantity of a substance in a mixture, you could use the technique of colorimetry, by allowing the substance to bind with colour forming chromo gens. The difference in colour results in the difference in the absorption of light, which is made use of here in this technique called colorimetry. Colorimeter uses different laws like Beer's law and Lambert's law.

KEY WORDS: Colorimetric sensing; Enzyme Linked Immunosorbent Assay (ELISA); point-of-care testing; lab-on-a-chip (LOC); Lab-on-Compact Disc (LOCD) platforms, immunosensors.

I.INTRODUCTION

Enzyme linked Immunosorbent Assay (ELISA) is a widely used clinical diagnostic tool used to detect a wide range of diseases from infectious diseases to cancer biomarkers. It is described as a precise, sensitive, versatile and quantifiable diagnostic method . Although there are various rapid screening test kits for antigen/antibody detection, they have lower sensitivity and specificity compared to sandwich ELISA assays . As an example, a conventional dengue ELISA test from Standard Diagnostics Inc. has a sensitivity of 98.8% and a specificity of 99.2%, whereas the rapid test kit from the same company has a sensitivity of 94.2% and a specificity of 96.4% according to data from the Standard Diagnostics product specifications. However, conventional ELISA tests are time-consuming, need specialized laboratory equipment and significant expertise to carry out. Hence, currently, they are unfeasible to apply in rapid testing and point-of-care diagnosis. For this reasons, researchers are trying to miniature the entire ELISA procedure on Lab-on-a-Chip (LOC) or Lab-on-Compact Disc(LOCD) platforms. The main applications of Elisa Reader are it is widely used in food and laboratory industry. We can detect the virus present in 96 samples using Elisa well plate. It is used to quantitative estimation of serum components as well as glucose, proteins and other various biochemical compounds. They are used by the food industry and by manufactures of paints and textiles. They are used to test for water quality and also used to determine the concentrations of plant nutrients.

A. Elisa Plate

The equipment of ELISA consists of a micro- plate which consists 96 wells. The wells are arranged in the form of 8 rows and 12 columns. By this arrangement we can detect 96 samples at a time. Marked on one side alphabetically and numerically on the other side. Elisa Plate is shown in Figure 1.

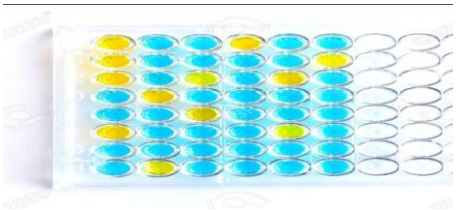

Figure 1: Elisa Plate

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B. Liquid crystal display(16x2)

A liquid crystal display (LCD) is a thin, flat display device made up of numbers of colour or monochrome pixels arrayed in front of a light source or Reflector. Liquid crystal screen display contains different control lines like EN (Enable) it is used to tell the LCD that you are sending data, RS (Register Select), R/W (Read/Write). And the logical status on control lines are like when EN (enable) is 0 it access to LCD disabled, if EN (enable) is 1 it access to LCD enabled. When R/W is 0 then it writes data to LCD, if R/W is 1 then it reads data from LCD. When RS (register select) is 0 is used for instructions, if RS (register select) is l is used for character. LCD is shown in Figure 2.

Figure 2: Liquid crystal display(16x2)

C. Arduino UNO

Arduino is a microcontroller-based open source electronic prototyping board which can be programmed with an easy-to-use Arduino IDE and it consists of both a physical programmable circuit board anda piece of software, or IDE and it uses a simplified version of C++. Arduino UNO is the most popular boards in the Arduino family. Arduino board can be powered through AC-to-DC adapter or a battery. Arduino UNO has 6 analog pins and 14 digital pins out of these 14 digital pins 6 pins can be used as PWM (pulse-width modulation) pins that are indicated with tilde symbol. Microcontroller used on UNO board is ATMEGA 328 shown in figure 3.

Figure 3: Arduino UNO

D. Light Source

A Light emitting diode (LED) is a semiconductor light source that emits light when current flows through it. Electrons in the semiconductor recombine with electron holes releasing energy in the form of photons. The colour of the light (corresponding to the energy of the photons) is determined by the energy required for electrons to cross the band gap of the semiconductor. White light is obtained by using multiple semiconductors or a layer of light-emitting phosphor on the semiconductor device. LED is shown in figure 4.

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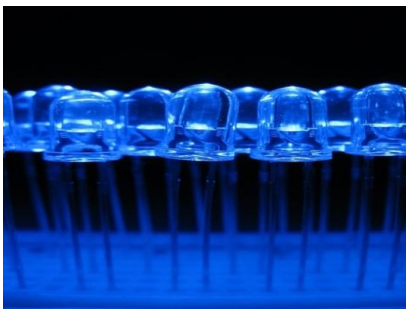

Figure 4: LED's

E. Optical Sensor

A photoresistor (acronymed LDR for Light decreasing Resistance, or light-dependent resistor, or photo-conductive cell) is an active component that decreases resistance with respect to receiving luminosity (light) on the component's sensitive surface. The resistance of a photoresistor decreases with increase in incident light intensity; in other words, it exhibits photoconductivity. A photoresistor can be applied in light-sensitive detector circuits and light-activated and dark-activated switching circuits acting as a resistance semiconductor. In the dark, a photoresistor can have a resistance as high as several megaohms (MΩ), while in the light, a photoresistor can have a resistance as low as a few hundred ohms. The figure 5 shows the Light dependent resistor.

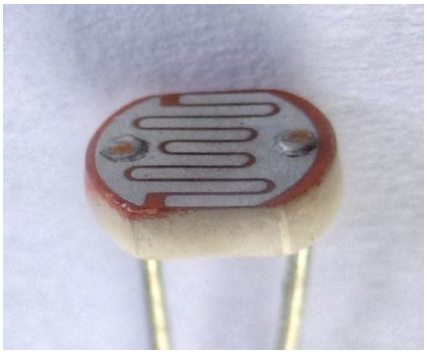

Figure 5: Optical Sensor (LDR)

F. Multiplexer

A multiplexer also known as a data selector, is a device that selects between a several number of analog and digital input signals and forwards it to a single output line. The 74HC151 is a 8-bit multiplexer with eight binary inputs (I0 to I7) had three select inputs (S0 ,S1 andS2) and an enable input (E). One of the eight binary inputs is selected by the select inputs and routed to the complementary outputs (Y and Y). A HIGH on E forces the output Y LOW and output Y HIGH. Inputs also include clamp diodes that enable the use of current limiting resistors to interface inputs to voltages in excess of V_{CC}. Figure 6 shows the Pin Configuration.

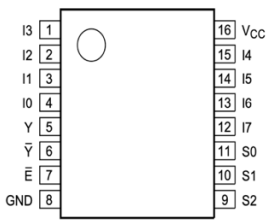

Figure 6: Pin Configuration of 74HC151

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G. Decoder

A binary decoder is a combinational circuit that converts binary information from the n coded inputs to a maximum of 2^n unique outputs. They are used in a wide variety of applications, including data multiplexing and data demultiplexing, seven segment displays.

The reverse of the digital demultiplexer is the digital multiplexer. The 74HC137 is a 3-to-8 line decoder, demultiplexer with latches at the three address inputs (An). The 74HC137 essentially combines the 3-to-8 decoder function with a 3-bit storage latch. When the latch is enabled (LE = LOW), the 74HC137 acts as a 3-to-8 active LOW decoder. When the latch enable (LE) goes from LOW-to-HIGH, the last data present at the inputs before this transition, is stored in the latches. Further address changes are ignored as long as LE remains HIGH. The output enable input (E1 and E2) controls the state of the outputs independent of the address inputs or latch operation. All outputs are HIGH unless E1 is LOW and E2 is HIGH. The 74HC137 is ideally suited for implementing non-overlapping decoders in 3-state systems and strobed (stored address) applications in bus oriented systems. Figure 7 shows the Pin configuration of 74HC137 decoder.

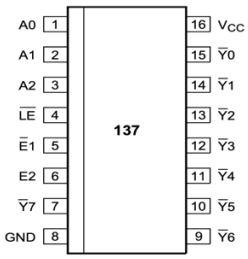

Figure 10: Pin Configuration of 74HC137

II. LITERATURE SURVEY

The enzyme-linked immunosorbent assay "ELISA" developed in recent years represents a significant addition to existing serologicaltols. Encouraging preliminary results obtained through its application to a number of parasitic diseases during the last two years indicate the value of further investigations and trials which will permit a true evaluation. Although the technique is easy to perform and quite sensitive, there are certain problems to be solved before it becomes widely usable. In the present Memorandum the technical details are given and the advantages and shortcomings of the procedure are discussed. Present applications and future prospects are reviewed.

III.METHODOLOGY

A) System Design

The design of this Elisa Reader comprised of main parts namely microcontroller, multiplexer and demultiplexer. The objective of is to illustrate the wave nature of light and the relationship between color and light absorbed.

A LED of certain wavelength is used in the design since the ELISA test result using different solutions like H2SO4 solution, blood sample, plasma and serum . H2SO4 have the highest sensitivity at this wavelength. The light is passed through the sample. Demultiplexer is used 96 LED's to glow simultaneously. The light intensity is read by the photodiode sensor i.e LDR.LDR'S are connected to the multiplexer for use of many number of LDR'S. The output of the sensor is digitized by an Analog to Digital converter (ADC). The results are then displayed on a Liquid Crystal Display (LCD). The microcontroller records each light intensity reading and then all the samples are recorded. The block diagram of Elisa Reader using 96-well plate is shown figure 11 below.

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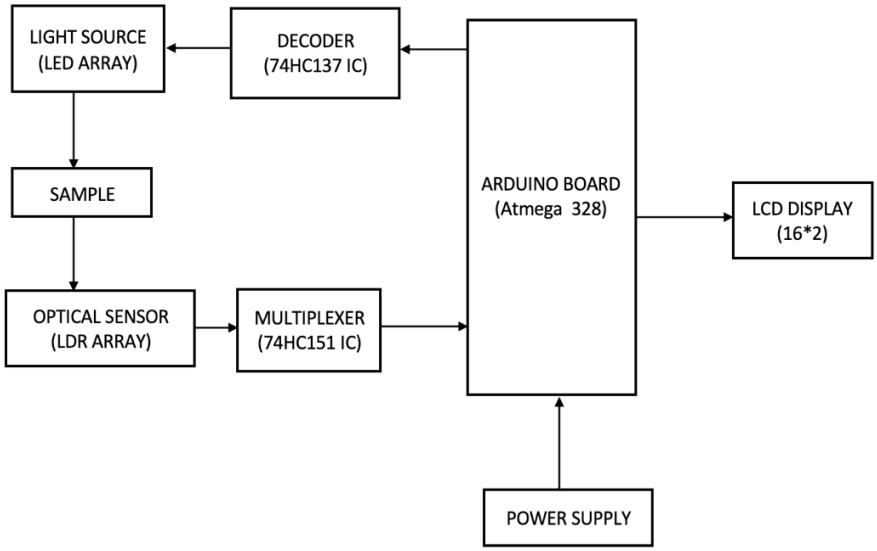

Figure 11: Block diagram

IV RESULTS

In summary, we have developed a Enzyme-Linked Immunosorbent Assay (ELISA) detection system using 96 well plate. The ELISA reader developed is evaluated to have clinical sensitivity of 95.2% and specificity of 100%. Since the device is portable, low cost and can be easily manufactured, it is well suited for point-of-care diagnosis, especially in resource-poor settings. However, for the qualitative results, used in actual clinical diagnosis, our device has been shown to be very accurate and precise. This device can used for various disease ELISA detection systems, it can be easily adapted to suit various colorimetric ELISA tests. We can not only test human blood samples but can test animal blood samples also using this colorimetric ELISA test.

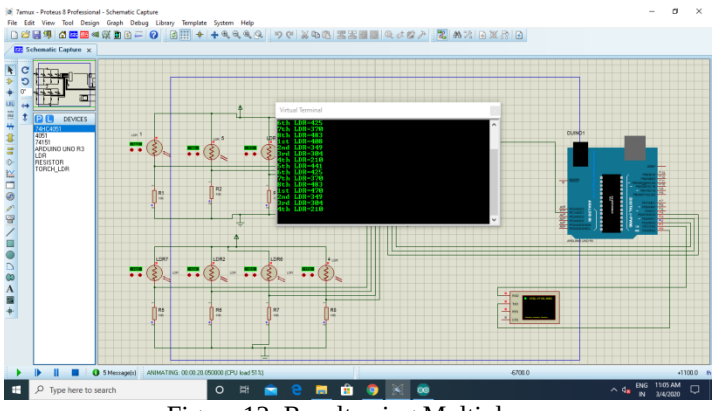

Figure 12: Result using Multiplexer

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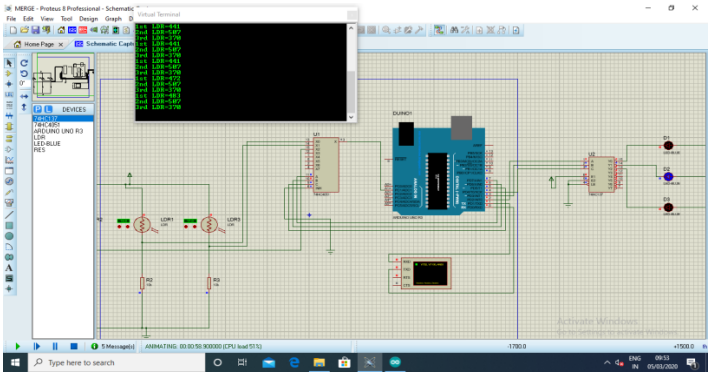

Figure 13: Result using Decoder

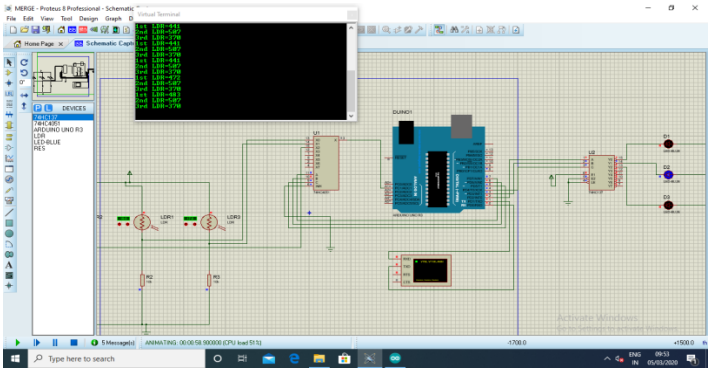

Figure 14: Result using both Multiplexer and decoder

V.CONCLUSION AND FUTURE WORK

The project " Elisa Reader using 96-well plate" is designed as a system such that to give complete solution for testing many number of samples at a time. This project is to illustrate the wave nature of light and the relationship between colour and light absorbed.

This project uses Elisa plate which has 96 well plates and can test many number of samples at a time and can detect the disease present in it. The LED is turned ON and passes some amount of light in the sample. The optical sensor detect the intensity of light present in the sample and send amount of light detected by the optical sensor. In this project Light Dependent Resistor is used as an optical sensor. The LDR sends the information to the microcontroller which is Atmega 328 embedded on the Arduino development board. Then the microcontroller send the respective control signals to the LCD.

ELISA tests can also be used to test the animal blood samples. It can also be to used to test any kind of virus like corona can also be detected using this ELISA.

By extending this proposed project by implementing and interfacing the GSM module, keypad and connecting it to the internet server this prototype can be used to receive the automatic information after the testing of a particular samples.

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