CHAPTER 1

INTRODUCTION

Every year more than 40% of the road accident patients die due to loss of blood even after the medication is provided. The reason behind these deaths is the methods which are used to determine the blood group and other blood elements. These methods are needed to be completed in a proper lab and a highly skilled pathologist is required to conduct the procedures. Hence, it requires a period of time to analyze the blood. This delay in determination of blood group can put injured patients a severe condition and may result in death. The current methods used requires human intervention to conduct the procedures, which may exhaust the person analyzing the blood sample and is prone to have a human error.

Blood is a non-Newtonian fluid is highly viscous because it contains constituents like platelet, plasma, white blood cells and more importantly Hemocrit. As per many research blood has a dynamic viscosity of 5.5 centi-poise.

The methods which are used to determine the blood group uses slides and tubes made of materials like plastic, fiber, glass, and ceramic. To analyze the blood, the blood sample has to go through numerous procedures and requires different type of chemicals for the analysis. Because of these chemicals and high viscous property of blood, the mixture gets stuck on the slides or tubes. These slides and glass tubes are discarded or to remove this mixture they are needed to be cleansed using chemicals like alcohol producing Non-degradable Bio-Waste.

The nature of this waste is hazardous for our environment. Many research states that, these ‘Non-degradable Bio-Waste’ can lead to diseases like breathing disorder, asthma, skin cancer and many more. Also, this Bio-waste cause serious problems like soil infertility and give rise to a waste land.

1.1 Constituents of Blood

In humans, blood is an opaque red fluid, freely flowing but denser and more viscous than water. The characteristic colour is imparted by hemoglobin, a unique iron-containing protein. Hemoglobin brightens in colour when saturated with oxygen (oxyhemoglobin) and darkens when oxygen is removed (deoxyhemoglobin).

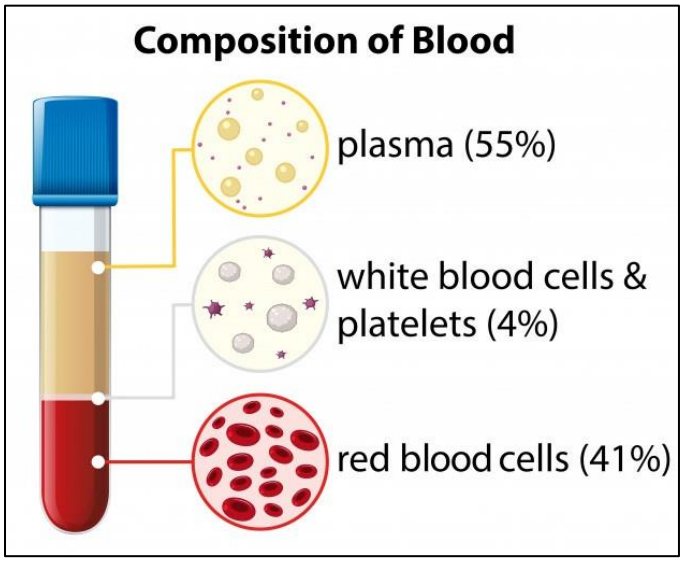


Fig. 1.1 Composition of Blood

For this reason, the partially deoxygenated blood from a vein is darker than oxygenated blood from an artery. The red blood cells (erythrocytes) constitute about 45 percent of the volume of the blood, and the remaining cells (white blood cells, or leukocytes, and platelets, or thrombocytes) less than 1 percent. The fluid portion, plasma, is a clear, slightly sticky, yellowish liquid. After a fatty meal, plasma transiently appears turbid. Within the body the blood is permanently fluid, and turbulent flow assures that cells and plasma are fairly homogeneously mixed.

The total amount of blood in humans varies with age, sex, weight, body type, and other factors, but a rough average figure for adults is about 60 millilitres per kilogram of body weight. An average young male has a plasma volume of about 35 millilitres and a red cell volume of about 30 millilitres per kilogram of body weight. There is little variation in the blood volume of a healthy person over long periods, although each component of the blood

is in a continuous state of flux. In particular, water rapidly moves in and out of the bloodstream, achieving a balance with the extravascular fluids (those outside the blood vessels) within minutes. The normal volume of blood provides such an adequate reserve that appreciable blood loss is well tolerated. Withdrawal of 500 millilitres (about a pint) of blood from normal blood donors is a harmless procedure. Blood volume is rapidly replaced after blood loss; within hours, plasma volume is restored by movement of extravascular fluid into the circulation. Replacement of red cells is completed within several weeks. The vast area of capillary membrane, through which water passes freely, would permit instantaneous loss of the plasma from the circulation were it not for the plasma proteins—in particular, serum albumin. Capillary membranes are impermeable to serum albumin, the smallest in weight and highest in concentration of the plasma proteins. The osmotic effect of serum albumin retains fluid within the circulation, opposing the hydrostatic forces that tend to drive the fluid outward into the tissues.

1.1.1 Constituents of Red Blood Cells (erythropoiesis)

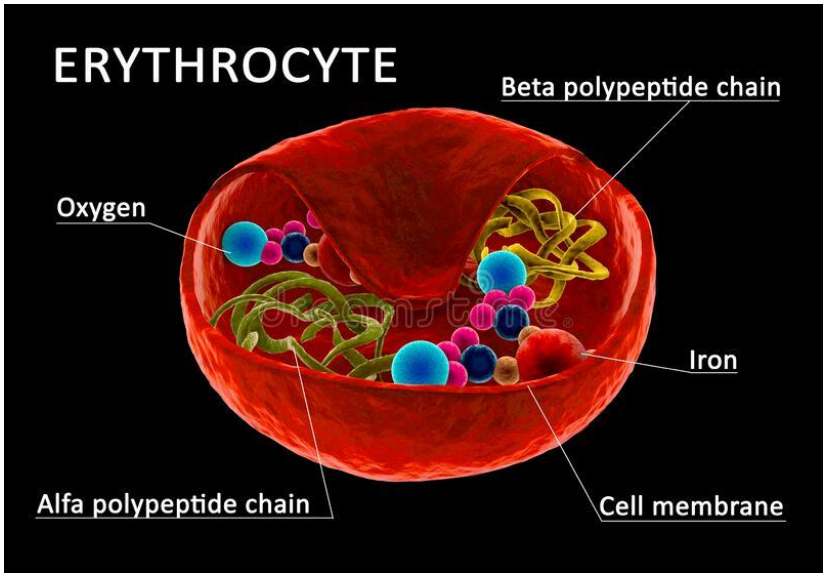


Fig. 1.2 Composition of RBC

The red blood cells are highly specialized, well adapted for their primary function of transporting oxygen from the lungs to all of the body tissues. Red cells are approximately 7.8 μm (1 μm = 0.000039 inch) in diameter and have the form of biconcave disks, a shape

that provides a large surface-to-volume ratio. When fresh blood is examined with the microscope, red cells appear to be yellow-green disks with pale centers containing no visible internal structures. When blood is centrifuged to cause the cells to settle, the volume of packed red cells (hematocrit value) ranges between 42 and 54 percent of total volume in men and between 37 and 47 percent in women; values are somewhat lower in children. Normal red blood cells are fairly uniform in volume, so that the hematocrit value is determined largely by the number of red cells per unit of blood. The normal red cell count ranges between four million and six million per cubic millimeter.

The red blood cell is enclosed in a thin membrane that is composed of chemically complex lipids, proteins, and carbohydrates in a highly organized structure. Extraordinary distortion of the red cell occurs in its passage through minute blood vessels, many of which have a diameter less than that of the red cell. When the deforming stress is removed, the cell springs back to its original shape. The red cell readily tolerates bending and folding, but, if appreciable stretching of the membrane occurs, the cell is damaged or destroyed. The membrane is freely permeable to water, oxygen, carbon dioxide, glucose, urea, and certain other substances, but it is impermeable to hemoglobin. Within the cell the major cation is potassium; in contrast, in plasma and extracellular fluids the major cation is sodium. A pumping mechanism, driven by enzymes within the red cell, maintains its sodium and potassium concentrations. Red cells are subject to osmotic effects. When they are suspended in very dilute (hypotonic) solutions of sodium chloride, red cells take in water, which causes them to increase in volume and to become more spheroid; in concentrated salt solutions they lose water and shrink.

When red cell membranes are damaged, hemoglobin and other dissolved contents may escape from the cells, leaving the membranous structures as “ghosts.” This process, called hemolysis, is produced not only by the osmotic effects of water but also by numerous other mechanisms. These include physical damage to red cells, as when blood is heated, is forced under great pressure through a small needle, or is subjected to freezing and thawing; chemical damage to red cells by agents such as bile salts, detergents, and certain snake venoms; and damage caused by immunologic reactions that may occur when antibodies

attach to red cells in the presence of complement. When such destruction proceeds at a greater than normal rate, hemolytic anemia results.

The membrane of the red cell has on its surface a group of molecules that confer blood group specificity (i.e., that differentiate blood cells into groups). Most blood group substances are composed of carbohydrate linked to protein, and it is usually the chemical structure of the carbohydrate portion that determines the specific blood type. Blood group substances are antigens capable of inducing the production of antibodies when injected into persons lacking the antigen. Detection and recognition of the blood group antigens are accomplished by the use of blood serum containing these antibodies. The large number of different red cell antigens makes it extremely unlikely that persons other than identical twins will have the same array of blood group substances.

1.1.2 Production of red blood cells (erythropoiesis)

Red cells are produced continuously in the marrow of certain bones. As stated above, in adults the principal sites of red cell production, called erythropoiesis, are the marrow spaces of the vertebrae, ribs, breastbone, and pelvis. Within the bone marrow the red cell is derived from a primitive precursor, or erythroblast, a nucleated cell in which there is no hemoglobin. Proliferation occurs as a result of several successive cell divisions. During maturation, hemoglobin appears in the cell, and the nucleus becomes progressively smaller. After a few days the cell loses its nucleus and is then introduced into the bloodstream in the vascular channels of the marrow. Almost 1 percent of the red cells are generated each day, and the balance between red cell production and the removal of aging red cells from the circulation is precisely maintained. When blood is lost from the circulation, the erythropoietic activity of marrow increases until the normal number of circulating cells has been restored.

In a normal adult the red cells of about half a liter (almost one pint) of blood are produced by the bone marrow every week. A number of nutrient substances are required for this process. Some nutrients are the building blocks of which the red cells are composed. For example, amino acids are needed in abundance for the construction of the proteins of the

red cell, in particular of hemoglobin. Iron also is a necessary component of hemoglobin. Approximately one-quarter of a gram of iron is needed for the production of a pint of blood. Other substances, required in trace amounts, are needed to catalyze the chemical reactions by which red cells are produced. Important among these are several vitamins such as riboflavin, vitamin B12, and folic acid, necessary for the maturation of the developing red cell; and vitamin B6 (pyridoxine), required for the synthesis of hemoglobin. The secretions of several endocrine glands influence red cell production. If there is an inadequate supply of thyroid hormone, erythropoiesis is retarded, and anemia appears. The male sex hormone, testosterone, stimulates red cell production; for this reason, red cell counts of men are higher than those of women.

The capacity of the bone marrow to produce red cells is enormous. When stimulated to peak activity and when provided adequately with nutrient substances, the marrow can compensate for the loss of several pints of blood per week. Hemorrhage or accelerated destruction of red cells leads to enhanced marrow activity. The marrow can increase its production of red cells up to eight times the usual rate. After that, if blood loss continues, anemia develops. The rate of erythropoiesis is sensitive to the oxygen tension of the arterial blood. When oxygen tension falls, more red cells are produced and the red cell count rises. For this reason, persons who live at high altitude have higher red cell counts than those who live at sea level. For example, there is a small but significant difference between average red cell counts of persons living in New York City, at sea level pressure, and persons living in Denver, Colo., more than 1.5 km (1 mile) above sea level, where the atmospheric pressure is lower. Natives of the Andes, living nearly 5 km (3 miles) above sea level, have extremely high red cell counts.

The rate of production of erythrocytes is controlled by the hormone erythropoietin, which is produced largely in the kidneys. When the number of circulating red cells decreases or when the oxygen transported by the blood diminishes, an unidentified sensor detects the change, and the production of erythropoietin is increased. This substance is then transported through the plasma to the bone marrow, where it accelerates the production of red cells. The erythropoietin mechanism operates like a thermostat, increasing or

decreasing the rate of red cell production in accordance with need. When a person who has lived at high altitude moves to a sea level environment, production of erythropoietin is suppressed, the rate of red cell production declines, and the red cell count falls until the normal sea level value is achieved. With the loss of one pint of blood, the erythropoietin mechanism is activated, red cell production is enhanced, and within a few weeks the number of circulating red cells has been restored to the normal value. The precision of control is extraordinary so that the number of new red cells produced accurately compensates for the number of cells lost or destroyed. Erythropoietin has been produced in vitro (outside the body) by the technique of genetic engineering (recombinant DNA). The purified, recombinant hormone has promise for persons with chronic renal failure, who develop anemia because of a lack of erythropoietin.

1.2 Blood Type Grouping

Blood types are based on the markers (specific carbohydrates or proteins) or antigens on the surface of red blood cells (RBCs). Two major antigens or surface identifiers on human RBCs are the A and B antigens. Another important surface antigen is called Rh. Blood typing detects the presence or absence of these antigens to determine a person's ABO blood group and Rh type.

People whose red blood cells have A antigens are in blood group A, those with B antigens are group B, those with both A and B antigens are in group AB, and those who do not have either of these markers are in blood group O.

If the Rh protein is present on the red blood cells, a person's blood type is Rh+ (positive); if it is absent, the person's blood is type Rh- (negative).

Our bodies naturally produce antibodies against the A and B antigens that we do not have on our red blood cells. For example, a person who is blood type A will have anti-B antibodies directed against the B antigens on red blood cells and someone who is type B will have anti-A antibodies directed against the A antigens. People with type AB blood have neither of these antibodies, while those with type O blood have both.

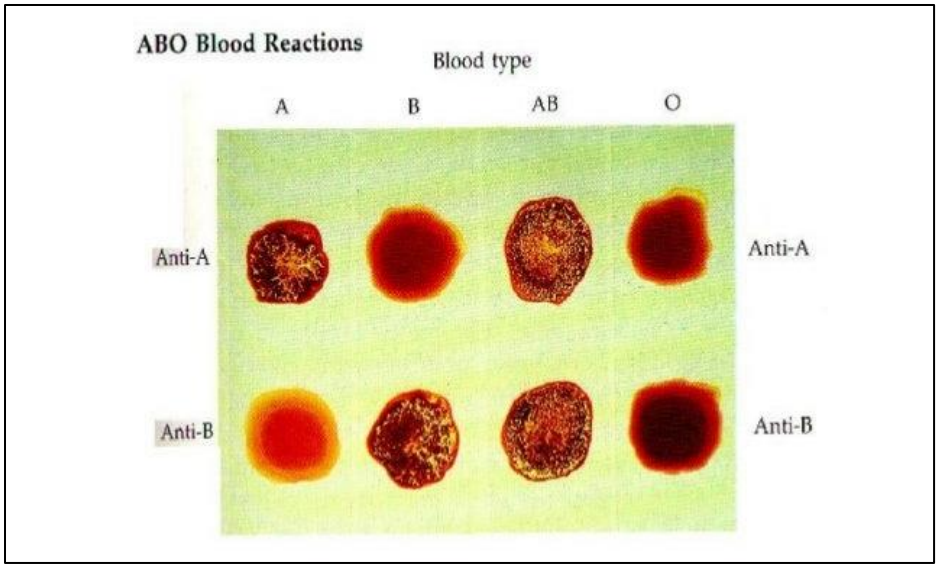


Fig. 1.3 Blood Antibodies in Anti-A and Anti-B solution

These antibodies are useful for determining a person's blood type and help determine the types of blood that he or she can safely receive (compatibility). If a person who is group A with antibodies directed against the B antigen, for example, were to be transfused with blood that is type B, his or her own antibodies would target and destroy the transfused red blood cells, causing severe, potentially fatal complications. Thus, it is critical to match a person's blood type with the blood that is to be transfused.

Unlike antibodies to A and B antigens, antibodies to Rh are not produced naturally. That is, Rh antibodies develop only after a person who does not have Rh factor on his or her red blood cells (Rh negative) is exposed to Rh positive red blood cells. This can happen during pregnancy or birth when a Rh-negative woman is pregnant with a Rh-positive baby, or sometimes when a Rh-negative person is transfused with Rh-positive blood. In either case, the first exposure to the Rh antigen may not result in a strong response against the Rh-positive cells, but subsequent exposures may cause severe reactions.

1.2.1 Types of Blood Grouping

a) Tube and slide methods:

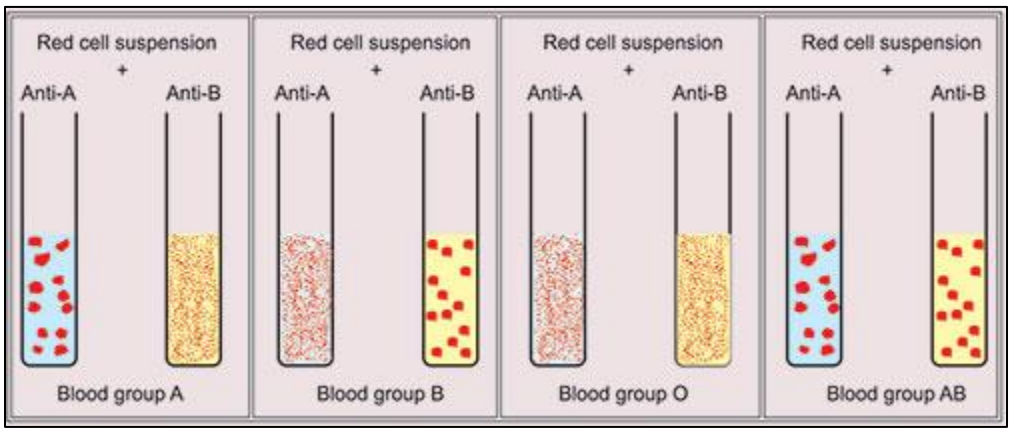


Fig. 1.4 Tube type Blood Grouping method

Blood typing can be performed using test tubes, microplates, or blood typing slides. The tube method involves mixing a suspension of red blood cells with antisera (or plasma, for reverse grouping) in a test tube. The mixture is centrifuged to separate the cells from the reagent, and then resuspended by gently agitating the tube. If the antigen of interest is present, the red blood cells agglutinate, forming a solid clump in the tube. If it is absent, the red blood cells go back into suspension when mixed. The microplate method is similar to the tube method, except rather than using individual test tubes, blood typing is carried out in a plate containing dozens of wells, allowing multiple tests to be performed at the same time. The agglutination reactions are read after the plate is centrifuged.

Antibody screening and identification can also be carried out by the tube method. In this procedure, the plasma and red cells are mixed together in a tube containing a medium that enhances agglutination reactions, such as low ionic strength saline (LISS). The tubes are incubated at body temperature for a defined period of time, then centrifuged and examined for agglutination or hemolysis; first immediately following the incubation period, and then after washing and addition of anti-human globulin reagent, likewise, may be performed by the tube method; the reactions are read immediately after centrifugation in the immediate spin crossmatch, or after incubation and addition of AHG in the full crossmatching procedure.

The slide method for blood typing involves mixing a drop of blood with a drop of antisera on a slide. The slide is tilted to mix the cells and reagents together and then observed for agglutination, which indicates a positive result. This method is typically used in under-resourced areas or emergency situations; otherwise, alternative methods are preferred.

b) Column Agglutination:

Column agglutination techniques for blood compatibility testing (sometimes called the "gel test") use cards containing columns of dextran-polyacrylamide gel. Cards designed for blood typing contain pre-dispensed blood typing reagents for forward grouping, and wells containing only a buffer solution, to which reagent red blood cells and plasma are added, for reverse grouping. Antibody screening and crossmatching can also be carried out by column agglutination, in which case cards containing anti-human globulin reagent are used. The gel cards are centrifuged (sometimes after incubation, depending on the test), during which red blood cell agglutinates become trapped at the top of the column because they are too large to migrate through the gel.

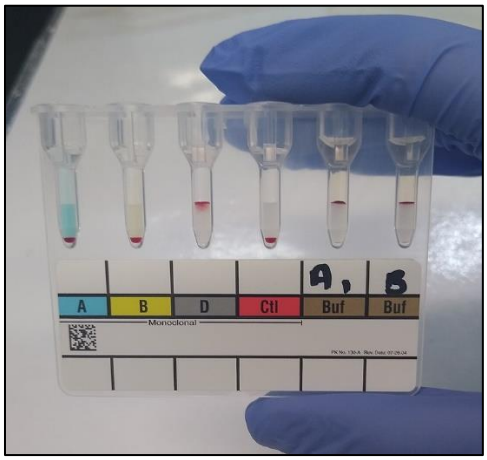


Fig. 1.5 Column Agglutination method

Cells that have not agglutinated collect on the bottom. Therefore, a line of red blood cells at the top of the column indicates a positive result. The strength of positive reactions is scored from 1+ to 4+ depending on how far the cells have travelled through the gel. The gel test has advantages over manual methods in that it eliminates the variability associated with manually re-suspending the cells and that the cards can be kept as a record of the test.

The column agglutination method is used by some automated analyzers to perform blood typing automatically. These analyzers pipette red blood cells and plasma onto gel cards, centrifuge them, and scan and read the agglutination reactions to determine the blood type.

c) Solid-phase assay:

Solid-phase assays (sometimes called the "antigen capture" method) use reagent antigens or antibodies affixed to a surface (usually a microplate). Microplate wells coated with anti-A, -B and -D reagents are used for forward grouping. The test sample is added and the microplate is centrifuged; in a positive reaction, the red blood cells adhere to the surface of the well. Some automated analyzers use solid phase assays for blood typing.

d) Genotyping:

Genetic testing can be used to determine a person's blood type in certain situations where serologic testing is insufficient. For example, if a person has been transfused with large volumes of donor blood, the results of serologic testing will reflect the antigens on the donor cells and not the person's actual blood type. Individuals who produce antibodies against their own red blood cells or who are treated with certain drugs may show spurious agglutination reactions in serologic testing, so genotyping may be necessary to determine their blood type accurately. Genetic testing is required for typing red blood cell antigens for which no commercial antisera are available.

The AABB recommends RhD antigen genotyping for women with serologic weak D phenotypes who have the potential to bear children. This is because some people with weak D phenotypes can produce antibodies against the RhD antigen, which can cause hemolytic disease of the newborn, while others cannot. Genotyping can identify the specific type of weak D antigen, which determines the potential for the person to produce antibodies, thus avoiding unnecessary treatment with Rho(D) immune globulin. Genotyping is preferred to serologic testing for people with sickle cell disease, because it is more accurate for certain antigens and can identify antigens that cannot be detected by serologic methods.

Genotyping is also used in prenatal testing for hemolytic disease of the newborn. When a pregnant woman has a blood group antibody that can cause HDN, the fetus can be typed

for the relevant antigen to determine if it is at risk of developing the disease. Because it is impractical to draw blood from the fetus, the blood type is determined using an amniocentesis sample or cell-free fetal DNA isolated from the mother's blood. The father may also be genotyped to predict the risk of hemolytic disease of the newborn, because if the father is homozygous for the relevant antigen (meaning having two copies of the gene) the baby will be positive for the antigen and thus at risk of developing the disease. If the father is heterozygous (having only one copy), the baby only has a 50% chance of being positive for the antigen.

1.3 METHODOLOGIES

1.3.1 Microfluidics

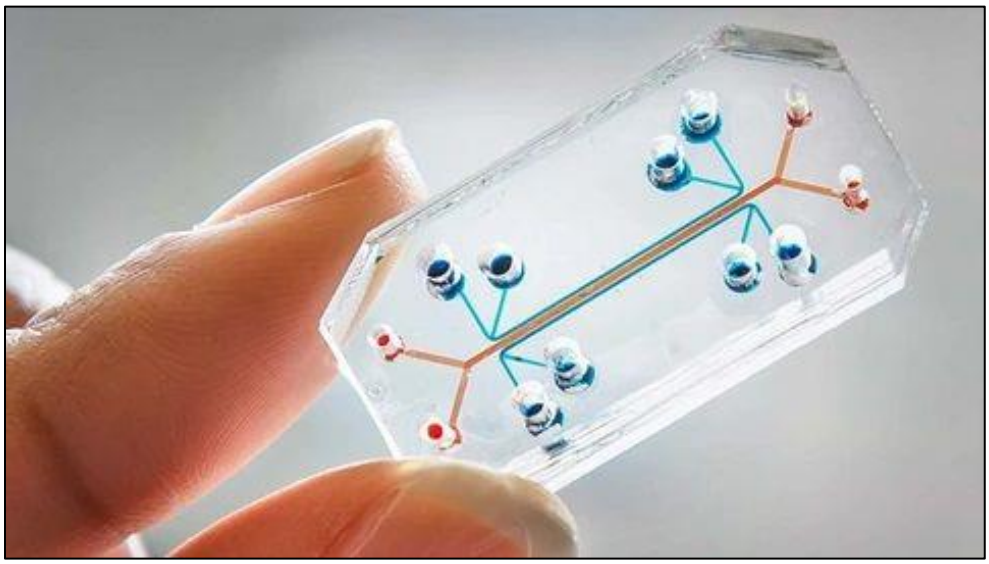


Fig. 1.6 Microfluidic Chip

Microfluidics is both the science which studies the behavior of fluids through micro-channels, and the technology of manufacturing microminiaturized devices containing chambers and tunnels through which fluids flow or are confined.

Microfluidics deal with very small volumes of fluids, down to femtoliters (fL) which is a quadrillionth of a liter. Fluids behave very differently on the micrometric scale than they do in everyday life: these unique features are the key for new scientific experiments and innovations.

A method called photolithography, which was initially created for the purpose of creating small features on circuits in the semiconductor industry, is used to create microfluidic systems. The process of photolithography is used to transfer the geometric shapes found on a mask to the surface of an appropriate substrate. It employs specialized polymers such as PDMS (Polydimethylsiloxane) that respond to particular light wavelengths to imprint the desired geometric patterns on a substrate.

The compatibility of PDMS with cells is the main advantage over conventional materials used in microelectronics. For cell biology applications such as sorting or patterning cells and proteins, cells-based biosensors, and coculture studies, microfluidic devices were developed in the late 1990s.

1.3.2 Image processing

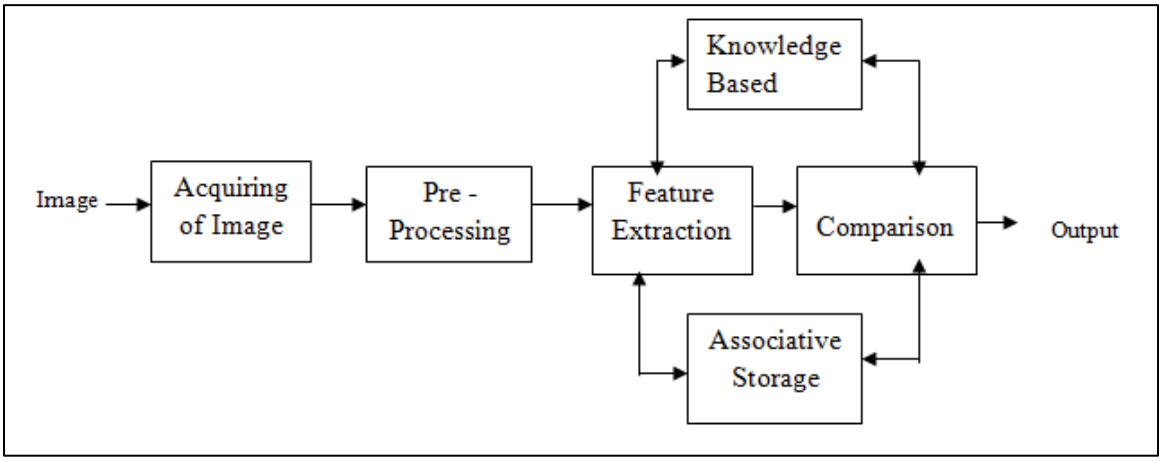


Fig. 1.7 Image Processing process flow chart

Image processing is a method to perform some operations on an image, the main operation is going to perform on image is Computer Vision, the computer vision is sub part or method of Artificial Intelligence also include the machine learning, in order to get an enhanced image or to extract some useful information from it. It is a type of signal processing in which input is an image and output may be image or characteristics/features associated with that image. Computer vision is an interdisciplinary scientific field that deals with how computers can gain high level understanding from digital images or videos. Nowadays,

image processing is among rapidly growing technologies. It forms core research area within engineering and computer science disciplines too.

Image processing basically includes the following three steps:

- Importing the image via image acquisition tools.
- Analyzing and manipulating the image.
- Output in which result can be altered image or report that is based on image analysis.

There are two types of methods used for image processing namely, analogue and digital image processing. Analogue image processing can be used for the hard copies like printouts and photographs. Image analysts use various fundamentals of interpretation while using these visual techniques. Digital image processing techniques help in manipulation of the digital images by using computers. The three general phases that all types of data have to undergo while using digital technique are pre-processing, enhancement, and display, information extraction.

For the using of digital image processing, need to be use a computer and an associated programming platform to implement digital image processing. Many programming platforms are available, but for the high performance the python is very efficient work on it. Python is also most preferred programming platform, many third-party image processing, and visualization libraries are available for python.

CHAPTER 2

LITERATURE REVIEW

David Nobes, Md Ashker Rashid, Shadi Ansari [1]

Capillary filling of non-Newtonian fluid is one of the complicated scenarios. Whether the case is underfilling of epoxy encapsulant in electronic packaging, blood flow or the viscoelastic flow through porous media. In such scenarios fluid is non-Newtonian and shear dependent viscosity plays a major role in the capillary filling process. Since most of the fluids used in practice has a non-Newtonian characteristic such as blood, polymeric solution, DNA in such cases the functional relationship between viscosity and capillary filling needs to be understood. In this capillary filling of non-Newtonian fluid is analyzed experimentally and theoretically. The objective is to examine the capillary filling process for both Newtonian and non-Newtonian fluid in capillary channel.

Non-mechanical pumping in microfluidic devices has emerged as a promising option for the pumping of biomolecules. Capillary filling due to dominant surface forces at the micro-scale is one such non-mechanical pumping approach. The ability of the pumping due to the capillary filling process is dictated by the surface energy of the channel surface and the physical properties of the liquid. The primary limitation for such surface tension driven flow is the induced drag caused by the inherent viscosity of the liquid and to determine the magnitude of this drag force, due to viscous drag, knowledge of velocity profile across the channel is of utmost priority. The capillary imbibition process is an intrinsically transient process and, in this study, the commonly adopted notion of fully developed velocity profile is experimentally verified. In such flows three different regimes along the flow can be found, i.e., surface forces dominant regime–behind the air-liquid interface, developing flow regime–where the viscous forces and surface forces are comparable to each other, and the developed regime–where the viscosity dictates the flow dynamics

Theoretical analysis of capillary process has been well studied and understood. The autonomous pumping ability allowed capillary filling process as a promising option as a passive pumping in microfluidic devices. Capillary pumping is also used to design cooling

system in electronic packaging and delivery system of μ-TAS (micro total analysis system) in the area of medicine and biology and to produce nanowires and nanorods in microfabrication designs. From theoretical point of view capillary filling process is due to the abundance of the surface forces or the surface energy and wettability of the substrate. The ability or pumping power of this autonomous pumping is mainly dictated by the physical and interfacial properties. Several theoretical approaches have been developed so far to predict the imbibition of liquid due to capillary action. In general, the driving force is balanced against the resistive force which is the viscous drag due to the inherent physical properties of the liquid. The knowledge of the velocity profile across the channel is an utmost requirement to quantify this viscous drag. In most of the cases the fully developed velocity profile is assumed to obtain the magnitude of the viscous drag. In this study, the assumption of fully developed velocity profile is verified against the experimental observation. The experimental analysis is further extended for non-Newtonian fluids and the velocity profile development in such cases is analyzed experimentally.

Franz L. Dickert, Adnan Mujahid [2]

Karl Landsteiner is the one who found the ABO Blood Grouping. There are different scientific names of each blood constituents like RBC called as Erythrocytes, WBC called as Leukocytes. In general, the practice of analyzing RBCs to identify the nature of antigen present in a blood sample is named BG typing. Principally, BG typing refers to a distinct chemical reaction between specific antibodies and BG antigens to monitor agglutination or blood clamping. In this way, the desired information about the nature of those antigens can be obtained.

There are strategic techniques or methods to find out the different types of blood group, another name for the same is Classical Strategies in BG typing.

Slide Method

The slide test is relatively the least sensitive method among others for BG determination but due to its prompt results it is very much valuable in emergency cases. In this method a glass slide or white porcelain supports is divided into three parts, as for each part, a drop

of donor or recipient blood is mixed with anti A, anti B, anti D separately. The agglutination or blood clumping pattern can be visually observed from which the ABO and rhesus D (RhD) type of blood can be determined. The test completes in 5 – 10 minutes and is inexpensive, which requires only a small volume of blood typing reagents. the test cannot be conducted for weakly or rarely reactive antigens form which the results are difficult ro interpret, and additionally a low titer of anti A or anti B could lead to false positive or false negative results. Although the slide test is useful for outdoor blood typing, it is not reliable enough for completely safe transfusion

Tube Test

In comparison with slide test the tube test is more sensitive and reliable, therefore it can be used conventionally for blood transfusion. In this method both forward (cell) as well as reverse (serum) grouping is carried out. The forward grouping suggests the presence or absence of A and B antigens in RBCs, whereas reverse grouping indicates the presence or absence of anti A and anti B in serum. In forward grouping, blood cells are placed in two test tubes along with saline as a diluent media, and then in drop, these tubes are subjected to centrifugation for few minutes, and the resultant matrix is gently shaken the observing agglutination.

Microplate Technology

Among classical methods, microplate technology is a further step towards more sensitive and fast blood typing analysis with the feasibility of automation. In this technique, both antibodies in blood plasma and antigens on RBCs can be determined. Typical microplates consist of a large number of small tubes that contain a few µL of reagents, which are treated against the blood samples. Following centrifugation and incubation, the subsequent agglutination can be examined by an automatic read out device. The microplate technique was first introduced in early 1950s; however, since then, considerable developments have been made in the design to improve the performance

Column/Gel Centrifugation

Column agglutination technology or gel centrifugation is a relatively modern approach that has gained substantial interest in ABO blood grouping, as it intends to establish a standard procedure for quantifying cell agglutination. Here, the column is made of small microtubes that contains gel matrix to trap agglutinates. Blood serum or cells are mixed with anti-A, anti-B and anti-D reagents in microtubes under controlled incubation and centrifugation. The gel particles trap the agglutinates, whereas non-agglutinated blood cells are allowed to pass through the column. The analysis time can be reduced by using glass beads in place of gel material, since in this way, faster centrifugation speeds can be achieved, which leads to rapid results. This technology is sensitive, straightforward and relatively easy to operate for less trained personnel.

Beckman Coulter, [3]

BLOOD GROUPING REAGENTS

BLOOD GROUPING REAGENTS	
Anti-A	**REF 17301**
Anti-A	**REF 27301**
Anti-B	**REF 17302**
Anti-B	**REF 27302**
Anti-A,B (Murine Monoclonal)	**REF 17303**
Anti-D	**REF 17304**
Anti-D (PK 1)	**REF 17305**
Anti-D (PK 2)	**REF 17304**
Anti-E	**REF 17306**
Anti-C	**REF 17309**
Anti-e	**REF 17319**
Anti-e	**REF B49558**
Anti-c	**REF 17314**
Anti-K (Human/Murine Monoclonal)	**REF 17315**

Table 2.1

The Anti -A, Anti -B, Anti -AB, Anti -D are used to determine the Rh(D) type. They are used to determine the absence or presence of Erythrocytic antigens A and/or B on the surface of human red blood cell.

The Anti D Reagents – Anti - D is used to detect the presence and type of Rh(D) antigen on the surface of human red blood cells.

The Anti -C, Anti – E, Anti – c, Anti – e, and Anti – K are used to determine the Rh(D) subgroups and kell phenotyping of human red blood cells. These reagents detect the presence of antigens C, E, c, e, and K on the surface of red blood cells.

ABO BLOOD GROUP SYSTEM

The determination of an ABO blood group is defined by demonstrating the presence or absence of antigens A and/or B on the surface of human red blood cells and by detecting the presence or absence of anti-A and/or Anti-B antibodies in the plasma. It is therefore appropriate to identify the erythrocyte antigens using known anti-A and anti-B, then to confirm the results by verifying the presence of the corresponding antibodies in the plasma from the test blood using known red blood cells A1 and B (reverse group). Additional testing of the red blood cells with Anti-A,B reagent facilitates the recognition of certain weak subgroups and is sometimes used as further confirmation of the reactions obtained with Anti-A and Anti-B reagents.

THE PRINCIPLE ANTIGENS AND ANTIBODIES OF THE ABO SYSTEM

ABO Blood Group	Antigen present on the red blood cells	Antibodies regularly present in the serum/plasma
O	neither A or B	anti-A and anti-B
A	A	anti-B
B	B	anti-A
AB	A and B	none

Table 2.2

Rh BLOOD GROUP SYSTEM

After the A and B antigens of the ABO blood group system, D is the most important blood group antigen in routine blood banking. Unlike antibodies of the ABO system, those of the

Rh system do not occur naturally in the serum but are most often the result of exposure to the antigen during pregnancy or through transfusion. The presence or absence of the D antigen is determined by testing the red blood cells with Anti-D. Agglutination indicates that the test cells are D positive. No agglutination indicates that the test cells are D negative. Approximately 85% of the white population and 94% of the black population are positive for the D antigen. The term "weak D" is used to describe forms of the D antigen that may not be agglutinated directly by Anti-D reagents. The red blood cells of donors are required to be tested for weak D before being classified as D negative 1,2. After the D antigen, the other most important antigens in the Rh system are C, E, c and e. These antigens are not as immunogenic as D, but may cause rapid destruction of red blood cells in the presence of the corresponding antibody. Positive results indicate the presence of the antigen, while negative results indicate the absence of the antigen on the red blood cells. It is significant to identify the presence of these antigens when selecting blood for transfusion to patients with these antibodies. Table 1 lists the five most common Rh antigens, the Weiner nomenclature and the approximate frequency of each antigen in the Caucasian population. Table 2 lists the most common patterns of reactions obtained and the most common genotypes.

Fisher-Race	Weiner	Caucasian %
D	Rh_0	85
C	rh'	70
E	rh''	30
c	hr'	80
e	hr""	98

Table fig 2.3 Rh Antigen Frequency

Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	Wiener	Fisher-Race
+	+	0	+	+	R^1r	CDe/cde
+	+	0	0	+	R^1R^1	CDe/CDe
0	0	0	+	+	rr	cde/cde
+	+	+	+	+	R^1R^2	CDe/cDE
+	0	+	+	+	R^2r	cDE/cde
+	0	+	+	0	R^2R^2	cDE/cDE
+	0	0	+	+	R^0r	cDe/cde
0	+	0	+	+	r'r	Cde/cde
0	0	+	+	+	r"r	cdE/cde

Table 2.4 Rh Common Patterns of Reaction and Probable Genotype

KELL Blood Group Systems

The most frequently encountered antibody in the Kell system is anti-K. The K(K1) antigen is strongly immunogenic, and anti-K is frequently found in the sera of transfused patients. A positive test indicates the presence of the K antigen, while a negative test indicates the absence of the K antigen on the red blood cells. Approximately 90% of Caucasian donors are K negative. It is significant to identify the K antigen when selecting blood for transfusion to patients with anti-K.

Geoff Daniels, Imelda Bromillow [4]

A blood group could be defined as, 'An inherited character of the red cell surface, detected by a specific alloantibody'.

Clinical Importance of Blood Groups

Blood groups are of great clinical importance in blood transfusion and in transplantation. In fact, the discovery of the ABO system was one of the most important factors in making the practice of blood transfusion possible. Many blood group antibodies have the potential to cause rapid destruction of transfused red cells bearing the corresponding antigen, giving rise to a haemolytic transfusion reaction (HTR), either immediately or several days after the transfusion. At their worst, HTRs give rise to disseminated intravascular coagulation, renal failure, and death. At their mildest, they reduce the efficacy of the transfusion.

Blood Group System

The international society of blood transfusion (ISBT) recognises 285 blood group antigens; 245 of these are classified into one of 29 blood group systems. Each blood group represents either a single gene or a cluster of two or three closely linked genes of related sequence and with little or no recognised recombination occurring between them. Consequently, each blood group systems are genetically discrete entity. The MNS system comprises three genes, Rh, Xg, and Chido/Rodgers, two genes each, and each of the remainder represents a single gene. Rh and MNS are the most complex systems, with 48 and 43 antigens, respectively; nine systems consist of just a single antigen

No.	Name	Symbol	No. of antigens	Gene name(s)	Chromo-some
001	ABO	ABO	4	*ABO*	9
002	MNS	MNS	43	*GYPA, GYPB, GYPE*	4
003	P	P1	1	*P1*	22
004	Rh	RH	49	*RHD, RHCE*	1
005	Lutheran	LU	19	*LU*	19
006	Kell	KEL	25	*KEL*	7
007	Lewis	LE	6	*FUT3*	19
008	Duffy	FY	6	*DARC*	1
009	Kidd	JK	3	*SLC14A1*	18
010	Diego	DI	21	*SLC4A1*	17
011	Yt	YT	2	*ACHE*	7
012	Xg	XG	2	*XG, MIC2*	X/Y
013	Scianna	SC	5	*ERMAP*	1
014	Dombrock	DO	5	*ART4*	12
015	Colton	CO	3	*AQP1*	7
016	Landsteiner–Wiener	LW	3	*ICAM4*	19
017	Chido/Rodgers	CH/RG	9	*C4A, C4B*	6
018	Hh	H	1	*FUT1*	19
019	Kx	XK	1	*XK*	X
020	Gerbich	GE	8	*GYPC*	2
021	Cromer	CROM	13	*CD55*	1
022	Knops	KN	8	*CR1*	1
023	Indian	IN	2	*CD44*	11
024	Ok	OK	1	*BSG*	19
025	Raph	RAPH	1	*CD151*	11
026	John Milton Hagen	JMH	1	*SEMA7A*	15
027	I	I	1	*GCNT2*	6
028	Globoside	GLOB	1	*B3GALNT1*	3
029	Gill	GIL	1	*AQP3*	9

Table 2.5 The blood Group Systems

Factors Affecting Antigen – Antibody Reactions

Temperature

The types of chemical bonds involved in a reaction are influenced by temperature. Polar (attractive) bonding in which electrons are interchanged between donor and acceptor molecules takes place in water-based media. The hydrogen bonds formed are exothermic and so stronger at lower temperatures. This type of bonding is normally associated with carbohydrate antigens

Non-polar molecules form hydrophobic bonds by expelling water and occur at higher temperatures. They are associated with protein antigens. The thermal range of an antibody is an indication of its clinical importance.

Time and Ionic Strength

In order to speed up the interaction of antigen and antibody, the ionic strength of the test milieu can be lowered. This means that solutions of saline containing less Na————— and Clions than normal isotonic salt solutions interfere less in the reaction between an antigen and antibody of complementary electrostatic charge.

Ph

The optimal pH for most antibodies of clinical importance has not been determined, however it is known that some antibodies react better outside the normal physiological pH range. Certain examples of anti-M react optimally at a pH of below 7 and in order to identify the specificity it may be necessary to acidify the serum. Under normal circumstances, a pH of about 7 is acceptable because red cells carry a negative charge and at pH 7–7.5 most antibody molecules bear a weakly positive charge. This enhances the attraction between the reactants during the first stage of agglutination or sensitization.

Antigen density

The number of antigen sites per cell and the accessibility of those antigens affects the optimal uptake of appropriate antibody. However, in conditions of antigen excess, the number of molecules of antibody bound on adjacent cells is relatively reduced, thereby producing less agglutination, or the formation of few, although large agglutinates

Direct and Indirect Agglutination

As the name suggests, this occurs as a direct result of an antigen– antibody interaction. Generally, the antibody is of the IgM immunoglobulin class and reactions take place optimally at low temperatures. Examples of IgG antibodies that can effect direct agglutination are known, such as anti-A and anti-B, and some IgG class anti-M. This is thought to be due to the structure carrying the appropriate antigen extending above the red

cell surface so that the distance between antigens on adjacent cells is less than 14 nM. This is the minimum distance between adjacent cells under normal conditions of ionic concentration, due to the mutual repulsive effect of the negative charge associated with red blood cells

Antibodies that cannot directly agglutinate cells are detected by means of enhanced test systems, notably the use of enzyme treatment of red cells or by the antiglobulin test, indirect agglutination.

G. Mohiuddin Mala, Dongqing Lit and J. D. Dale [5]

The paper discusses about the effects of the EDL at the solid-liquid interface on heat transfer and liquid flow through a microchannel between two parallel plates at constant temperatures are examined in this paper.

The Poisson-Boltzmann equation's approximate linear solution is used to describe the EDL field close to the solid-liquid interface. The equation of motion takes into account the electrical body force produced by the double layer field. The steady state flow is solved for by the motion's equation.

This paper discusses the effects of the EDL field and the channel size on the distribution of velocity, streaming potential, apparent viscosity, temperature distribution, and heat transfer coefficient.

The theories in this paper were used to for designing of the Blood Group Detection device

Dong Liu and Suresh V. Garimella [6]

In this paper, liquid flow in microchannels is studied numerically and experimentally. The experiments are conducted at Reynolds numbers ranging from 230 to 6500 in microchannels with hydraulic diameters of 244 to 974 m.

These microchannels' pressure drop is assessed, and it is also calculated by accounting for inlet and exit losses in global measurements. Flow visualization confirms the beginning of turbulence. Comparisons between numerical predictions and experimental measurements of pressure drop are made.

The findings of this study demonstrate that, for the range of dimensions taken into consideration, conventional theory can be used to accurately predict the flow behavior in microchannels. Comparisons between numerical predictions and experimental measurements of pressure drop are made.

The results of this study demonstrate that, for the range of dimensions taken into consideration, conventional theory can be used to accurately predict the flow behavior in microchannels.

The theories in this paper were used to prepare a mathematical model for the prototype to Dynamic flow parameters.

David W. Inglis et al. [7]

The paper discusses about the development of new microfluidic flow cytometer hereby reducing its cost and enhancing its functionalities. In order to reduce cost and complexity, microfluidic flow cytometers currently analyse significantly fewer parameters than conventional flow cytometry or fluorescence activated cell sorting (FACS). Microfluidic devices are required that can analyze more and/or new cell parameters with minimal and portable equipment.

For this purpose, the paper has presented the "hydrodynamic" cell size of the RBC's of blood, a novel and explicitly microfluidic parameter, and compares it with the forward scatter theory in traditional flow cytometry.

The amount of lateral displacement that cells experience as they pass through a 1.2 mm wide non-clogging array of microfabricated obstacles serves as a measure of the hydrodynamic size of the cell. Without lysing red blood cells, we demonstrate that the microfluidic device's size resolution is comparable to forward scatter in traditional flow cytometry. The device was used to quantify the number of activated lymphocytes in blood after exposure to staphylococcal enterotoxin B (SEB), and to distinguish healthy lymphocytes from malignant lymphocytes by size alone.

The results show that a microfluidic device can accomplish some of the measurement and separation functions of a flow cytometer at a lower cost and level of complexity.

From this paper the working theory the Blood Group Detection Device was determined.

Bernhard Sebastian and Petra S. Dittrich [8]

The discusses about the Capillary systems, which have contributed to the development of the basic laws of blood flow and its non-Newtonian properties throughout history.

The development of highly integrated lab-on-a-chip platforms that enable extremely accurate replication of the dimensions, mechanical properties, and biological complexity of vascular systems was sparked by the introduction of microfluidics technology in the 1990s.

Applications include the highly sensitive detection of pathological changes in platelets, white blood cells, and red blood cells as well as the evaluation of drug treatment effectiveness. Recent efforts have focused on the replication of more complex diseases like thrombosis or the development of microfluidics-based tests usable in a clinical setting.

These microfluidic disease models allow us to analyze the disease onset and progression as well as the identification of significant players and risk factors, which has produced a range of clinically significant findings.

From this paper, the nature of blood in microfluidic flow was obtained and used for designinig of the micro channels.

Melinda A. Lake et al. [9]

The fabrication protocols for creating master moulds and the finished polydimethylsiloxane (PDMS) device, as well as the testing of the finished microfluidic device, is discussed in this paper.

This paper's objective is to offer instructions to gain a better understanding of what is required to turn a microfluidic device idea into a finished product for testing.

AutoCAD design of a microfluidic device is presented. It also explains the primary fabrication steps and provides instructions for fabricating the finished microfluidic device using polydimethylsiloxane (PDMS) and the techniques employed to test the devices.

From this paper material and fabrication process for microfluidic part determined.

Adelina-Gabriela Niculescu et al. [10]

The paper discusses the principles of fluid dynamics, microelectronics, material science, physics, chemistry, and biology are all combined in the relatively new field of microfluidics.

According to the paper, various materials can be processed into miniature chips with microscale channels and chambers. Such platforms can be produced using a variety of techniques to achieve the desired size, shape, and geometry.

Microfluidic chips can be used for cell analysis, diagnosis, and culture as well as for the preparation of nanoparticles, drug encapsulation, delivery, and targeting, and cell analysis.

This paper discusses microfluidic technology in terms of the fabrication methods and platform materials that are currently available.

Ankita Dalvi, Hanu Kumar Pulipaka [11]

Based on the processing of digital images acquired during the slide test, a software is developed in image processing to determine the blood group during emergency situations without any error. The images obtained are then processed, occurrence of blood clumping is checked and accordingly the blood group is determined. Thus, using image processing techniques, this developed automated method will be useful in determining the blood group.

the software developed based on image processing techniques allows detection of agglutination on the slide through an image captured after mixing specific reagents and consequently the blood group of the patient is determined.

Mrs. G. Sangeetha Lakshmi, Ms. M. Jayashree. [12]

The proposed system aims to develop an embedded system which uses Image processing algorithm to perform blood tests based on ABO and Rh blood typing systems. The proposed system helps in reducing human intervention and perform complete test autonomously from adding antigens to final generation of the result. The proposed system aims at developing results in shortest possible duration with precision and accuracy along with storage of result for further references. Thus, the system allows us to determine the blood type of a person eliminating traditional transfusions based on the principle of the universal donor, reducing transfusion reactions risks and storage of result without human errors.

The detection of the composite organisms from a sample blood slide has been done via image processing techniques like threshold morphological operations . Errors can be occurred in these procedures if the detection of agglutinations is solemnly done with human eyes. Wrongly calculated blood group results in extreme situations in case of further diagnostics upon that decision. For determining the correct blood group we need an impeccable operation justified with logical and mathematical calculations and flawless image processing to detect residual errors that evade corrective procedures. Image segmentation is one of the most fundamental techniques of image processing. In segmentation, a bigger image is divided into a number of sub images.

While the algorithms run individually on the sub-divided images, the calculations occur more specifically and the result becomes more precise. There are several ways of image segmentation. Otsu method is one of them. Otsu is an automatic threshold selection region based segmentation method. Another Significant and important image processing technique is thresholding. Thresholding does binarization on any image. Some special thresholding techniques also does denoising. In some cases, some segmented image becomes cloudy and the important information which is needed to be extracted become complicated to retrieve. In such situations thresholding is very helpful . So, basically, thresholding techniques makes an image in black and white and it makes the image much clearer. One automated design was brought up where the researcher suggested the whole

test was done based on slide test for determining blood types and a software developed using image processing techniques. The image was processed by image processing techniques developed with the IMAQ Vision software from National Instruments.

Anushka Gulati, Rutu Patel, Vishal Ajith Mathew, M. Jagannath [13]

The image of the performed slide test is captured through a Raspberry Pi camera and the information about the obtained image is acquired by texture analysis using the gray-level co-occurrence matrix (GLCM) feature extraction method. Using this information, the SVM classification model determines the status of ABO and Rh antigens in the blood cells. Together, the ABO system and the Rh system provide the blood group of the sample. The method is established upon a dataset collected from a clinical laboratory. For the determination of the blood group, 40 samples of slide test results were processed, out of which 34 were detected correct, giving an accuracy of 85%. The proposed system seems to be accurately determining the ABO blood group without human intervention

The result is captured through a Raspberry Pi camera and goes through a series of pre-processing techniques namely RGB-to-grayscale image conversion followed by binarization. The pre-processed image is used for detecting edges using the canny edge detection method. This method is considered as an ideal edge detection technique for images that are corrupted with noise, reducing the possibility of detecting false edges, and provides sharp edges [5]. The derived image is then separated into three sub-images, taking into consideration that the sample is tested against three antibodies (Anti- A, Anti- B, Anti-D). The texture feature for each sub-image is extracted using the GLCM feature extraction method. This method is used for extracting second-order statistical texture features, namely contrast, correlation, homogeneity, and entropy [6]. These features are used as attributes to train the SVM classifier model to determine the presence/absence of agglutination. Therefore, each sub-image is classified into agglutinated or non-agglutinated class (labelled as 1 or 0), indicating the status of ABO and Rh antigens. Hence, using this information, the blood group is determined.

CHAPTER 3

PROPOSED DESIGN

3.1 Design Objectives

Some Design Objectives have been made, before designing the BGDD (Blood Group Detection Device)

I. Portability:

The process of determining the blood group is requires a skilled pathologist. Therefore, in case of emergency such as road accidents, pathologist is not available at the moment also performing the procedure is also not feasible. The BGDD should be designed light and compact, so that can be moved easily by means of hands in case of emergency.

II. Fully Automated:

The orthodox methods are tidies and requires human intervention, therefore, to eliminate this need the device should be designed in such a way that it runs completely without human intervention and give the blood group as an output.

III. Bio-waste reduction:

As discussed earlier in the report, the methods used to determine the blood group give away lot of bio-waste. Therefore, design of the BGDD should eliminate this problem by reusing some parts to determine the blood group.

3.2 Design consideration

To design the systems accurately, some design considerations were made. Such as:

I. Blood viscosity:

To reduce the viscosity of blood a solution named Ethylenediamine Tetra-acetic acid (EDTA) is added to it. Hence, after which it is assumed that blood have similar properties as water. Hence, becomes a Newtonian fluid which obeys the laws of fluid flow.

II. Pressure on the blood:

From the prototype model it was determined that the pressure of the 932.80 N/m^2 is enough to start the diluted blood flow through the 2 mm capillary.

III. Antigens Position:

From the prototype it is determined that the flow of any one fluid is enough to into another is enough to start the process of agglutination. Therefore for final product the antigen solutions are held stationary in the capsules shown in figure

3.3 Calculations

As the blood is considered as a Newtonian fluid, the flow of blood through the capillary of 2 mm can be calculated by the Bernoulli's theorem

To find the pressure P2 and other parameters a mathematical model using python was developed:

Mathematical model:

```
p1 = 932.80  # N/m2

d1 = 0.015  # m

d2 = 0.002   # m

z1 = 0.425 # m
```

```
z2 = 0.400 # m

A1 = ((22 / 7) / 4) * (d1 * d1)  # m2

A2 = ((22 / 7) / 4) * (d2 * d2)  # m2

g = 9.81  # m/s

rho = 1.224

rpm = 60

myu = 0.0055

v1 = (2 * 3.142) * (d1 / 2) * (rpm / 60)

print ("Pressure 1",p1)

print("Velocity 1 =",v1)

Q = A1 * v1 # m3 / s

print("Discharge =", Q)

v2 = Q / A2

print(("Velocity 2 ="), v2)

Re = (rho * d2 * v2) / myu

print("Renold's number =", Re)

f = 0.316 / (Re ** 0.25)

print("Darcy's friction factor =",f)

dp = (f * rho * v2 ** 2 * 60) / (2 * 2)

print("difference in pressure =", dp)

p2 = p1 - dp

print("Pressure 2",p2)
```

Results:

Pressure 1 932.8

Velocity 1 = 0.04713

Discharge = 8.331910714285715e-06

Velocity 2 = 2.6510625000000005

Renold's number = 1.1799638181818186

Darcy's friction factor = 0.303193516427265

Difference in pressure = 39.12303335894

Pressure 2 893.67696664106

CHAPTER 4

3D MODEL

The 3D modelling of the Blood Group Detection Device is done with the help of Solidworks 2021. While designing the device, some objectives were followed. Such as

a) Portability:

To make the device light and compact.

b) Fully Automated:

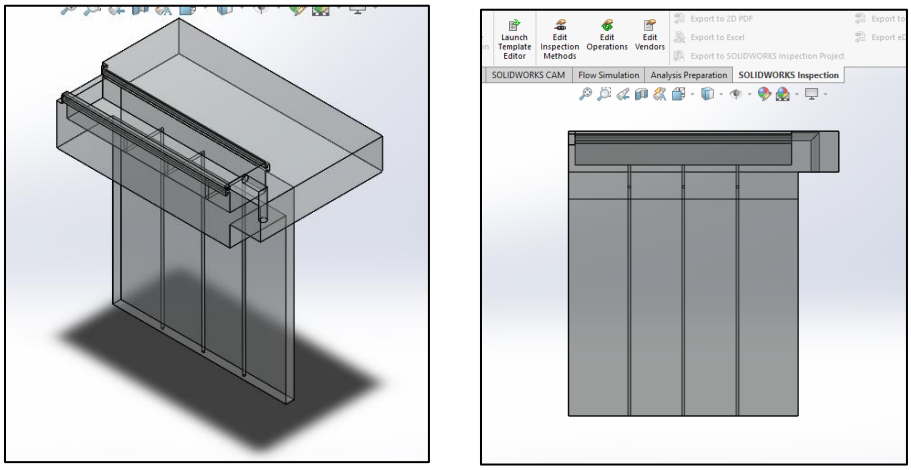
To eliminate human intervention in the process.

c) Bio-waste:

To reduce the biowaste produced compared to earlier methods.

4.1 Part Design

1 Main body:



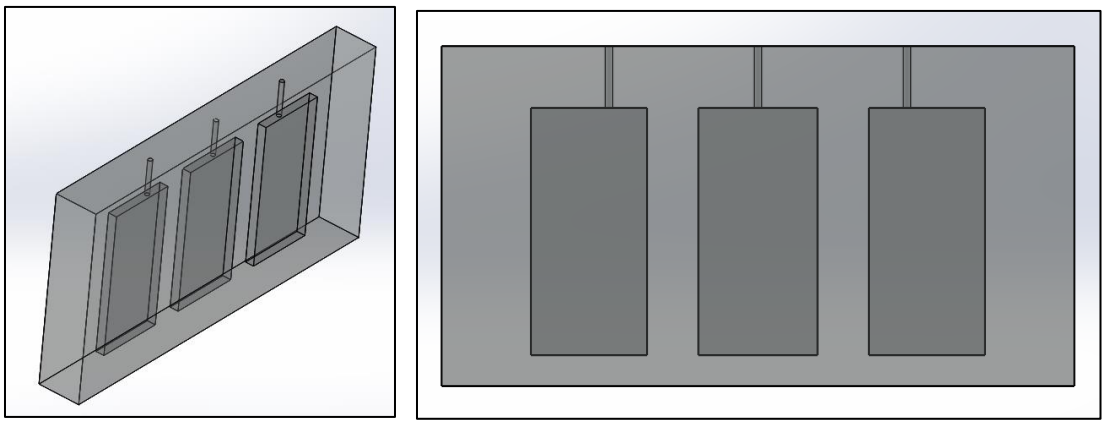
(a) (b)

Fig. 4.1 a) Isometric view and (b) Front view of Main body

The figure __ shows the isometric view and front of the main body. The main body has a cavity on top face to feed the diluted blood to system. The cavity has three small holes of

1 mm diameter at the bottom and one 8 mm hole on the side face as shown in the figure. The main body is made of PDMS(Polydimethylsiloxane).

2 Glass Capsule:



(a) (b)

Fig. 4.2 a) Isometric view and (b) Front view of Glass Capsule

The figure shows the isometric view of glass capsule which placed below the main body such that the hole on both main body and glass capsule matches. Also, the glass capsule has three cavities of 32x16x8 mm dimension which also a rubber bush at bottom end.

3 Raspberry Pie:

Fig. 4.3 Raspberry Pie

A Raspberry pie is used as a microcontroller to control and analyze the processes. It is fitted the case.

4 Adrucam:

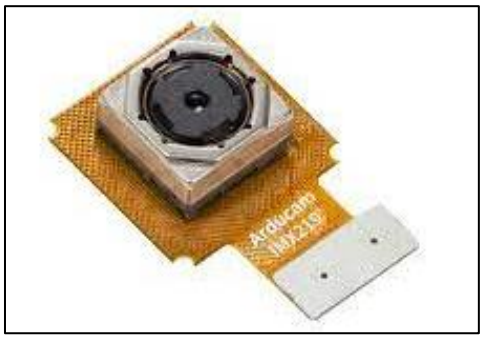


Fig. 4.4 Adrucam

Three small cameras are used of adrucam to capture the images.

5 Arducam multi camera adapter:

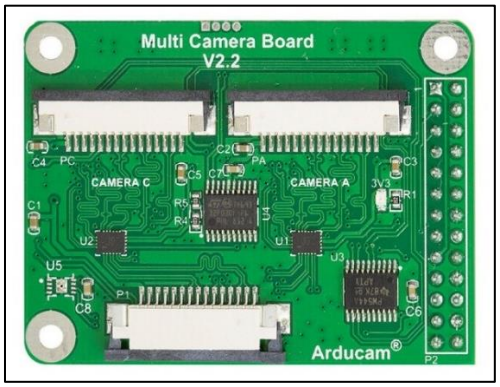


Fig. 4.5 Arducam multi camera adapter

This adapter is used to control all the cameras and provide input to the raspberry pie

6 Mini Air Pump:

Fig. 4.6 Mini Air Pump

The mini air pump supplies air to the upper cavity of main body.

7 LED Screen:

Fig. 4.7 LED Screen

It is used to show the results of process.

8 Case:

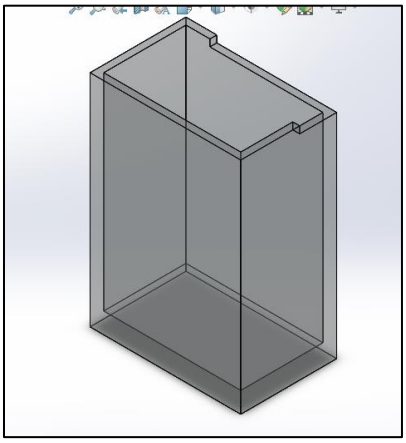


Fig. 4.8 Case

The case hold all the parts in it and acts a support member.

Fig. 2.3 shows the assembly of Blood Group detection device. It consists of eight components connected to each other. Fig 2.4 shows the main body of the device which has cavity on top face and a vertical slide which have 3 small holes of micro-dimensions. The holes from main body are connected to the detachable capsules, filled with antigen, of 32x16x8 mm dimension and the cavity is connected with Mini Air Pump by a small tube which blows air into the cavity. The main body is placed inside the case together with raspberry pie, Adrucam Multi camera adapter, three Adrucam's and a mini air pump as

shown in fig. 2.3. Also, all three Adrucam's are placed in such a way it captures image from each capsule.

Assembly design

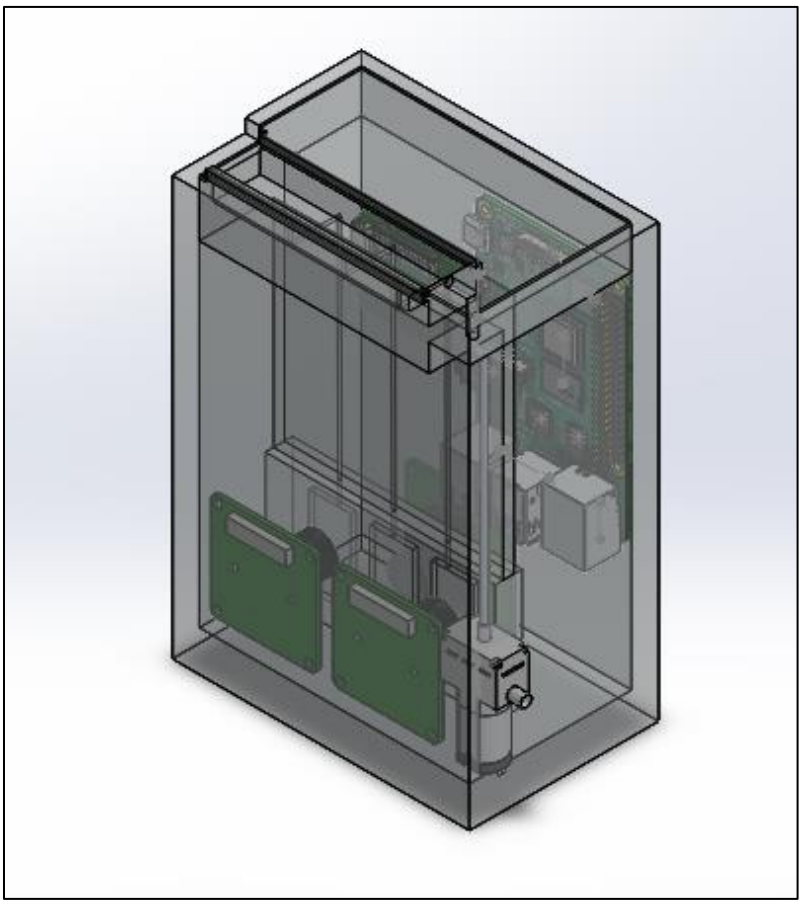


Fig. 4.9 Final Assembly of the Blood Group detection device

4.2BO Working of BGDD (Blood Group Detection Device)

The diluted blood is filled in the cavity present at the top face of main body and the cap is closed to make completely sealed cavity. The air is pumped into the cavity with the help of mini air pump. Because of the closed chamber the pressure rises and forces blood to flow through the hole provide vertically. Hence forcing pressured diluted blood into the capsules connected. Due to the pressure the diluted blood gets mixed with the antigen solutions and hence, forms the antibodies of blood. The images of these antibodies are

captured by the Adrucam's and then processed by the raspberry pie. After the evaluation the results are displayed on the LED screen.

2.3.4 Cost of Manufacturing:

Components	Material	Operation	Rate	Quantity	Cost
Main body	Silicone	Soft lithograpgy	70000	1	70000
Glass Capsule	SLS	3d printing	15000	1	15000
Raspberry pi	-	-	7000	1	7000
Adrucam	-	-	4953	3	14589
Adrucam Multi Camera Adapter	-	-	7	1	7
Mini Air Pump	-	-	170	1	170
LED Screen	-	-	82.50	1	82.50
Case	-	-	500	1	500
				Total	**107348.5/-**

Table 2.6 Cost of Manufacturing

CHAPTER 5

CFD ANALYSIS

CFD analysis for steady flow was carried out using Solidworks 2018 Flow Simulator The purpose of the CFD analysis was to verify the working of system for diluted blood. Main aim was to determine the change in pressure. Following figures shows the results of the simulation:

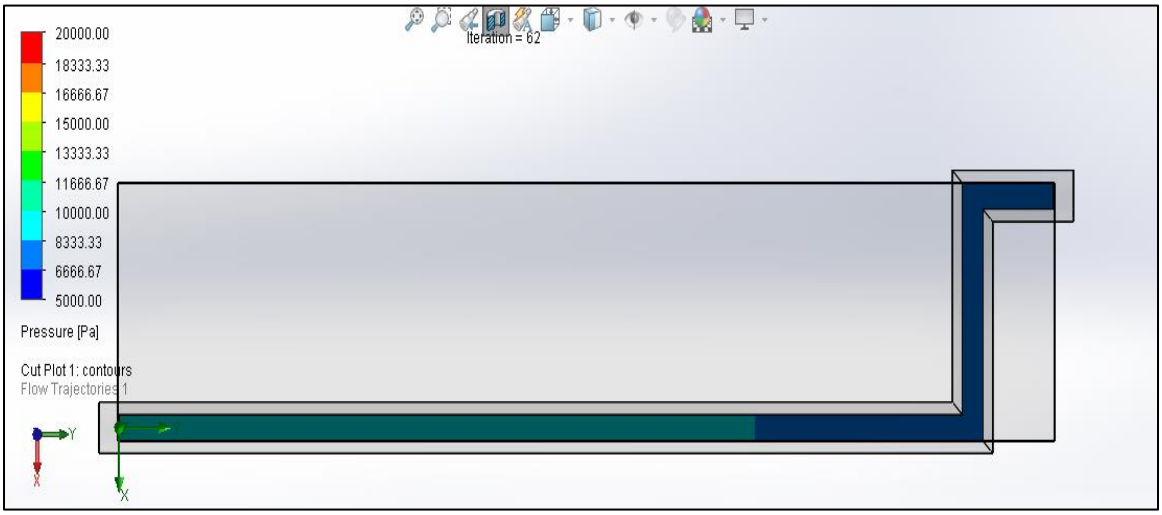


Fig. 5.1 Change in Pressure

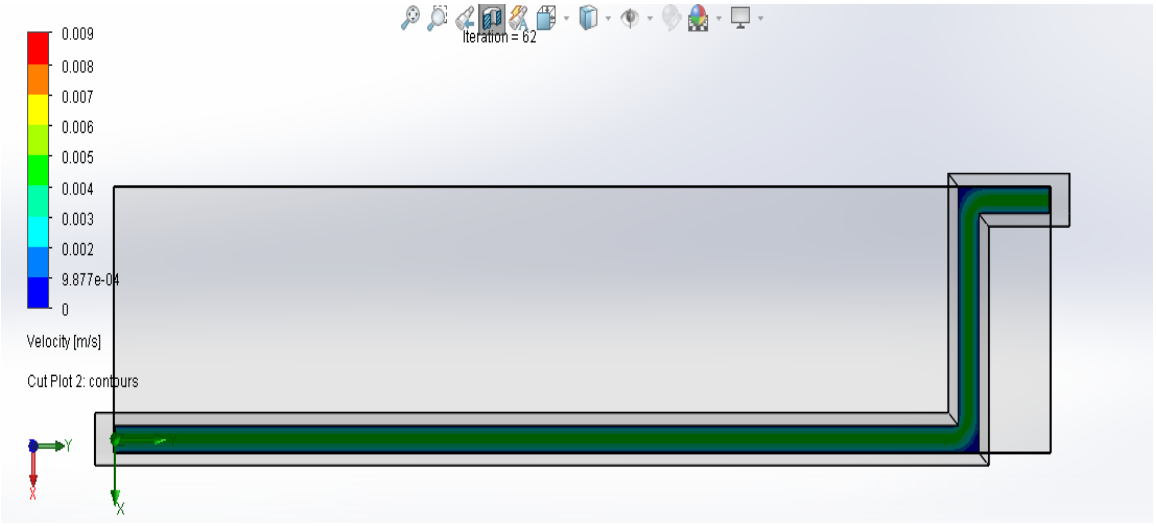


Fig. 5.2 Change in Velocity

5.1 Boundary layer conditions

The simulation was conducted on blood at 8956 pascal pressure. Fig 5.1, depicts the change in pressure at the end of the orifice. Through this simulation it was found at the exit of capillary tube the pressure was increased 8046 Pascal to 8932. Similarly, velocity of fluid also got increased from 0.002 m/s to 0.004 m/s.

5.2 Result and Discussion

The CFD analysis validates the design consideration that the fluid is considered Newtonian, as it obeys Bernoulli's theorem.

CHAPTER 6

PROTOTYPE MODEL

6.1 Prototype 1:

6.1.1 Apparatus

1. Three-way Connector – 1
2. Siring – 2
3. Silicone tube – 2 mm diameter
4. Diluted Blood – 2 ml
5. Antigens – 2 ml
6. Glass test tube

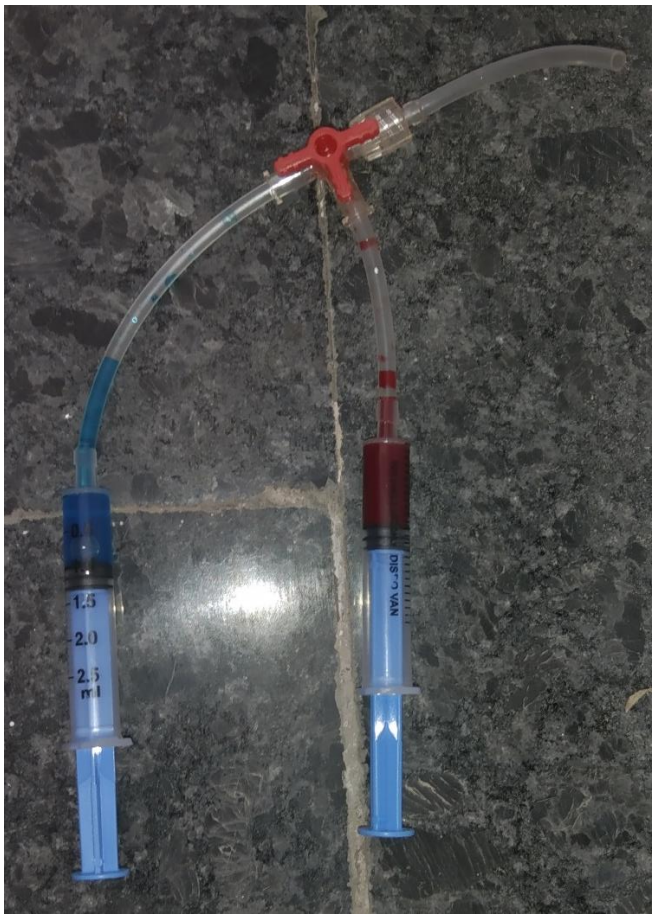


Fig. 6.1 Setup of the Experiment.

6.1.2 Construction

Three-way connector which has 3 ports attached of which the two ports are connected to the syringes containing Diluted Blood and Antigen respectively. Glass slide is placed below the third port, on which the mixture of diluted blood and antigen sample falls.

Two syringes filled with diluted blood and antigen solution respectively are connected to the inputs of the three-way connector.

6.1.3 Procedure

In the experiment, the syringes plunge diluted blood and antigen forcefully into three-way connector through the silicone tube and they get mixed with each other. As the blood and antigen are plunged from the syringe it increases its pressure which thereby reduces the time required for the Agglutination process of blood cells in the solution. The mixture is collected in the test tube from the third port and the sample is formed through which the blood group is analyzed.

6.1.4 Results and discussion

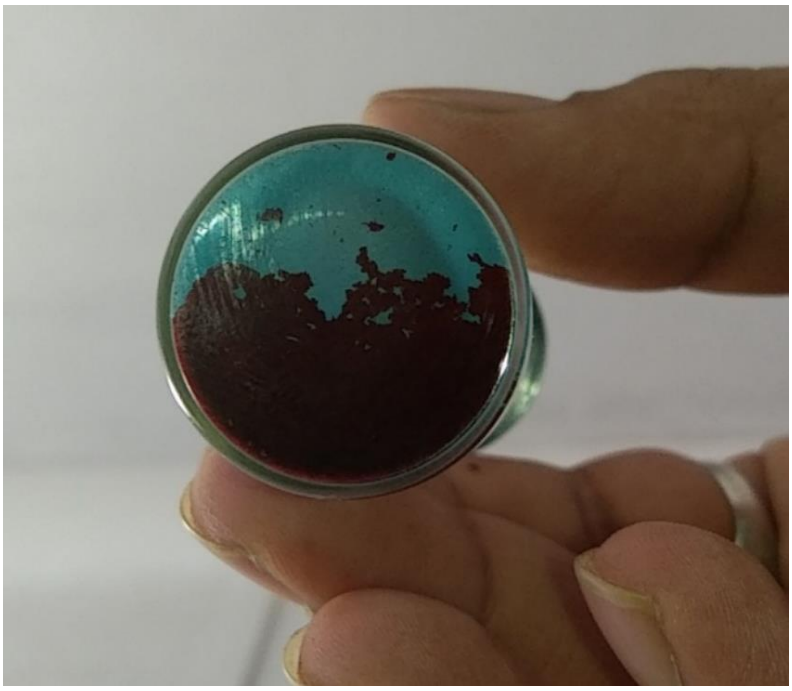


Fig. 6.2 Agglutination of blood in Anti-A solution

Fig. 6.3 Agglutination of blood in Anti-D solution

The Fig. 2.2 show the agglutination of blood cells in the anti-A and anti-D solution; through this it can be determined that the blood sample was of A+ group. Also, the time required for the agglutination of blood in Anti-A solution was 2 seconds and similarly in Anti-D solution it was 7 second.

6.1.5 Conclusion of experiment

From the experiment it can be concluded that adding pressure to both blood and antigen reduces the time required for Agglutination of Blood cells as compared to all previous methods i.e. 10 seconds in contrast to the previous methods which required 5 minute to determine the blood group. Hence, it can be stated that the time is the function of pressure.

Also, form the results of this experiments it is validated that, the pressure is directly proportional to the diameter of tube. Hence, it is proved that the use of microfluidics can give faster and accurate results.

6.2 Prototype 2

6.2.1 Objective

- To eliminate human intervention in the process.
- To study the methodologies.
- To check the programing for image processing.

6.2.2 Mathematical model

To find the optimum Speed (RPM) for stepper motor between 30 to 120 rpm, a mathematical model was prepared with the help of python

Following code was employed to find the optimum solution:

```
import matplotlib.pyplot as plt

import pandas as pd

d = 0.02

A = ((22 / 7) / 4) * d ** 2

I = 0.2  # Am

V = 5  # Volt

P = (I * V) / 1000  # Kw

N = pd.read_csv('Book1.csv')

T = ((P * 60) / (2 * (22 / 7) * N)) * 1000

V1=((22/7)*d*N)/60

F1 = 2 * (T / d)

F2 = 2 * F1

Fb = 0.981  # Weight of wooden block

Ft = F2 + Fb
```

```
Pr = (Ft / A) / 6

d1 = 0.015  # m

d2 = 0.002  # m

L=0.080

z1 = 0.425  # m

z2 = 0.400  # m

A1 = ((22 / 7) / 4) * (d1 * d1)  # m2

A2 = ((22 / 7) / 4) * (d2 * d2)  # m2

g = 9.81  # m/s

rho = 1.224

myu = 0.0055

Q = A1 * V1  # m3 / s

v2 = Q / A2

Re = (rho * d2 * v2) / myu

f = 0.316 / (Re ** 0.25)

h=((32*myu*v2*L)/(rho*g*d**2))

dp = (f * rho * v2 ** 2 * 60) / (2 * 2)

p2 = Pr - dp

#plt.plot(N, V1)

#plt.xlabel('RPM')

#plt.ylabel('Velocity')

#plt.plot(N, T)
```

```
#plt.xlabel('RPM')

#plt.ylabel('Torque(N.m)')

#plt.plot(N, Pr)

#plt.xlabel('RPM')

#plt.ylabel('Pressure (Pa)')

#plt.plot(V1, Q)

#plt.xlabel('Velocity(m/s)')

#plt.ylabel('Discharge(m3/s)')

#plt.plot(V1, Re)

#plt.xlabel('Velocity(m/s)')

#plt.ylabel("Reynold's No.")

#plt.plot(V1, Pr)

#plt.xlabel('Velocity(m/s)')

#plt.ylabel('Pressure(pa)')

#plt.plot(V1, p2)

#plt.xlabel('Velocity(m/s)')

#plt.ylabel('Pressure(pa)')

#plt.plot(V1, h)

#plt.xlabel('Velocity(m/s)')

#plt.ylabel('Frictional head loss')

plt.grid()

plt.show()
```

6.2.3 Result and discussion

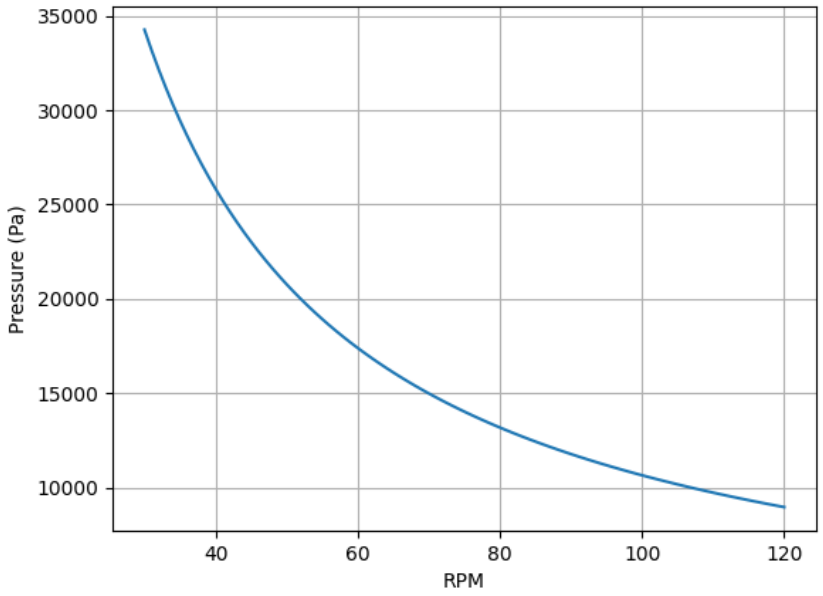


Fig. 6.4 Pressure vs RPM

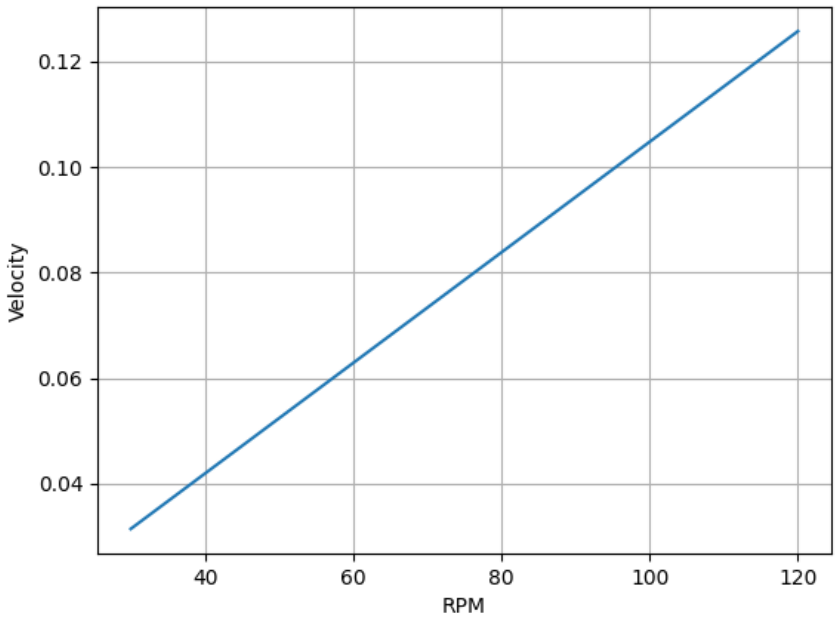


Fig. 6.5 Velocity vs RPM

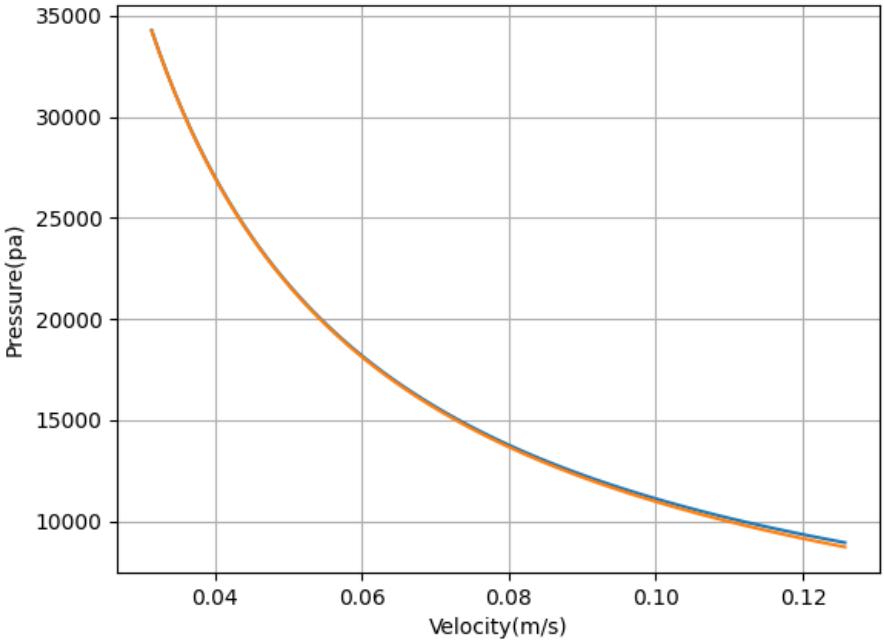


Fig 6.6 Pressure vs Veocity

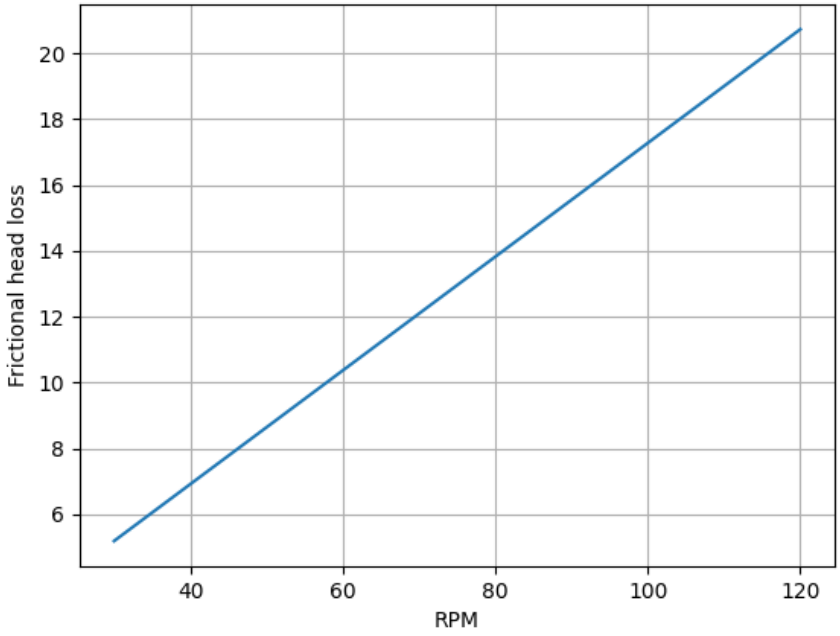


Fig 6.7 Frictional head loss vs RPM

The figure represent a graph of Speed (Rotation Per Minute) Vs Pressure acting on the fluid, which was generated at that Speed. From the graph at 60 rpm, the pressure was found to be 17460 Pascal which lies in an optimum range. Also, as shown in the fig , the velocity of fluid at 60 rpm was 0.065 m/s which is also in an optimum range.

Figure shows the variation in the pressure at inlet and outlet the pressure of 30 Pascal was found at 60 rpm. Also, the figure figure shows the frictional head loss during the fluid flow with respect to the RPM, at 60 rpm was 10.

From this mathematical model it was found that among the set of 30, 60, 90 and 120, 60 rpm is most optimum and is used for further designing.

6.3 Part Design

1. Wooden case:

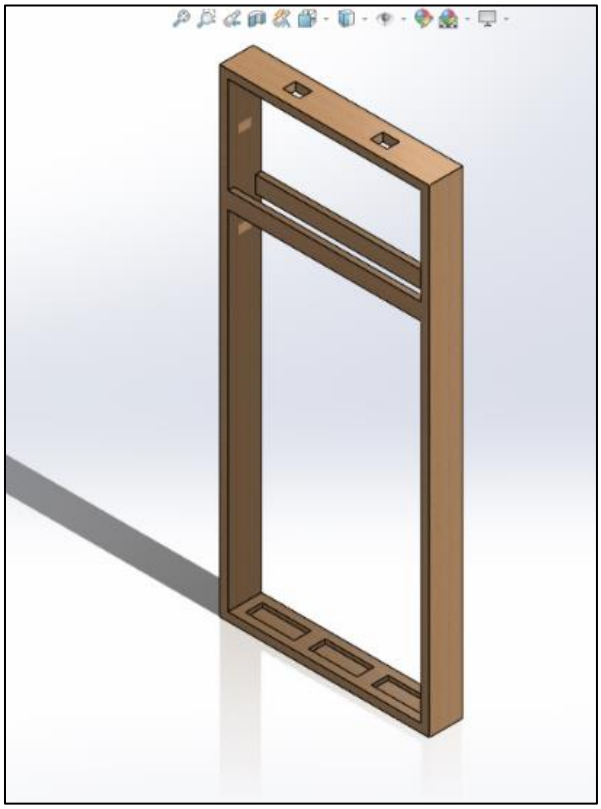


Fig. 6.8 Wooden case

The wooden case acts as a base support member of the system on which all the other part are mounted. It is made up of plywood.

2. Camera

Fig. 6.9 Webcam

The small camera is fitted at the bottom of the case to capture the image.

3. Test Tube

Fig 6.10 Test Tube

The 3 test tubes are used to collect blood and antigen mixtures respectively.

4. Syringe

Fig 6.11 Syringe

Six syringes are used to plunge the antigen solution and diluted blood

5. Arduino

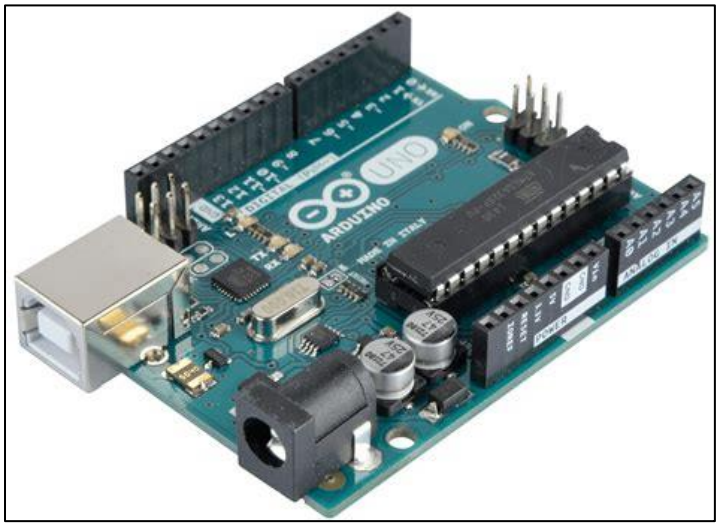


Fig 6.12 Arduino

The Arduino is used as a microcontroller to control the processes

6. Stepper Motor

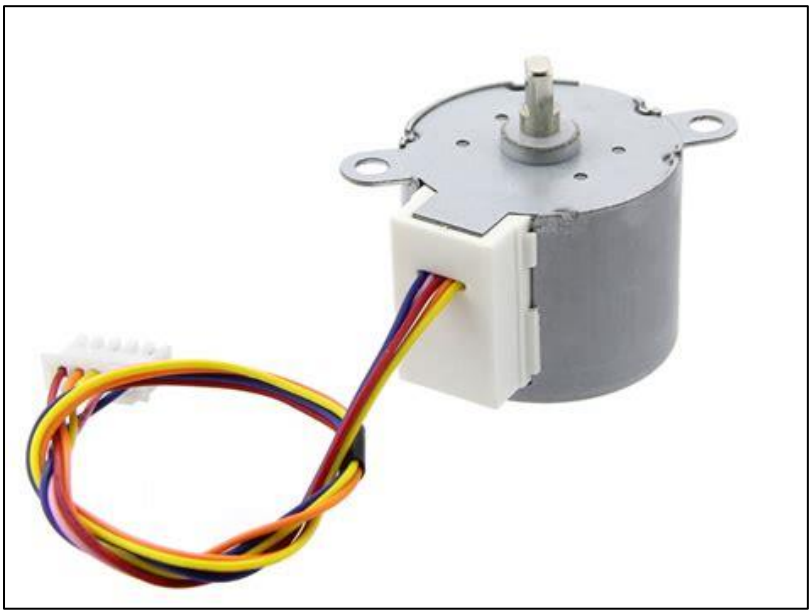


Fig 6.13 Stepper Motor

The stepper motor is used to give motion to the pinions

7. Rack and Pinion

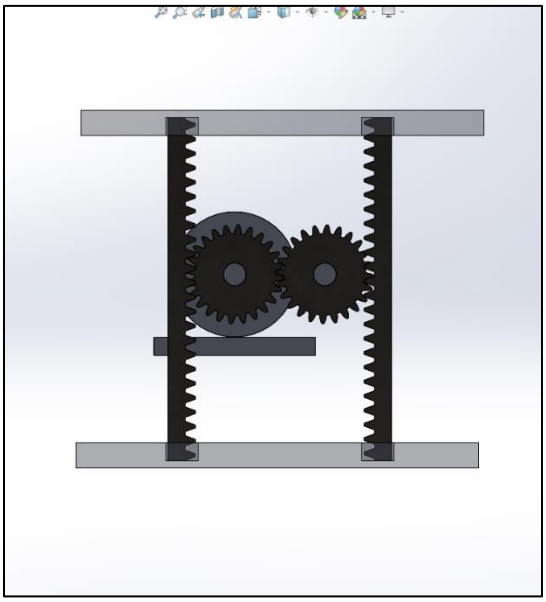


Fig 6.14 Rack and Pinion

The rack and pinion assembly are connected to plunger of syringe

8. Silicone tube

Fig. 6.15 Silicon Tube

The silicone tube is used to connect the syringe and three-way connector. Also, it delivers the mixture from the output of the three-way connector to the test tube.

9. Three-way

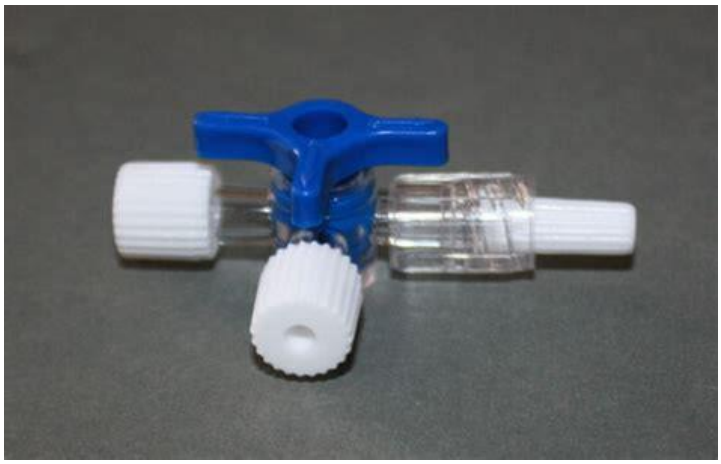
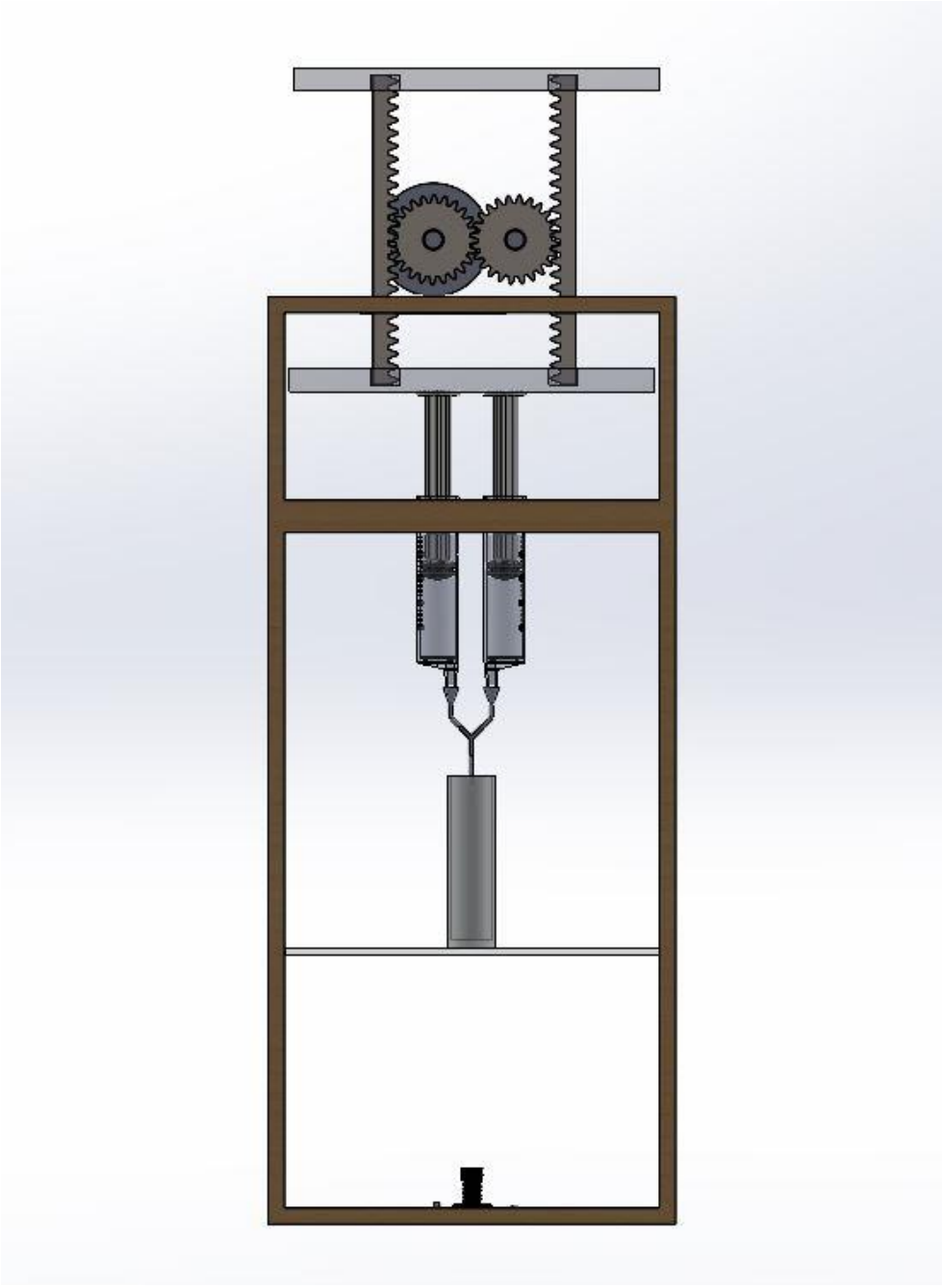


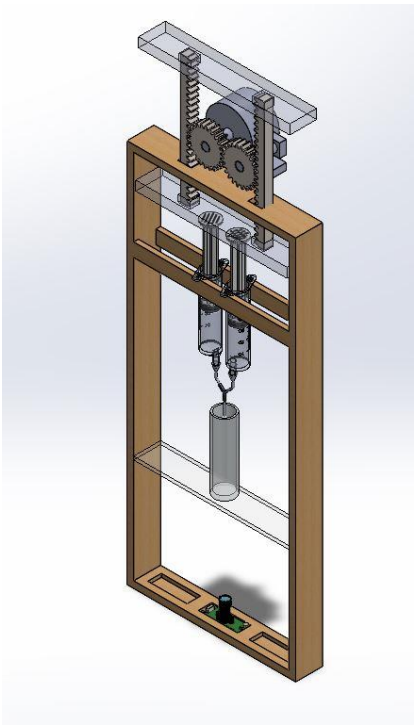
Fig 6.16 Three Way

The three-way connector is connected with two syringe and as a mixing agent of diluted blood and antigen solution.

6.4 Assembly Design



a)



b)

Fig 6.17 a) Front and b) Isometric view of Prototype

The prototype contains three test tubes which are placed at the bottom of the wooden case as shown in figure. Six syringes are hung over these test-tubes in the wooden case. Every two syringes are connected to two inputs of three-way with a silicone tube of 2mm inner diameter and the output is given to test-tube. Below the test tube a camera is placed. The plungers of the syringes are connected to a wooden block and this wooden block is connect to the rack and pinion assembly as shown in figure , which is driven by stepper motor.

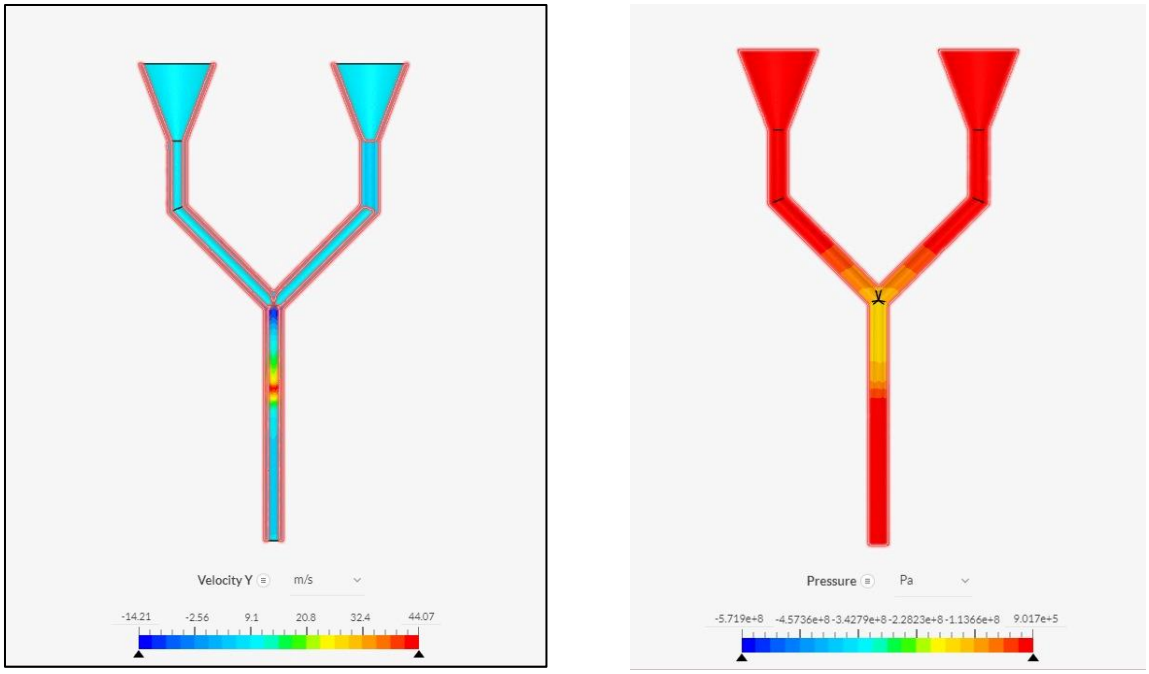
6.4.1 Working of Prototype:

As shown in figure, the syringes 1, 3 and 5 are filled with blood and syringes 2, 4 and 6 are filled with antigen A, B and D respectively. The stepper motor is maintained at the constant speed with the help of Arduino and the pinion starts rotating. When the pinion rotates, the rack moves in downward direction, hence pushing the wooden block. The plungers of syringe are attached to the wooden block which also moves in the same direction. Due to this movement the plunger pushes the blood and antigens to the inputs of

three-way where the blood is mixed with antigen and the Agglutination of blood according to its blood type. From the output of three-way this mixture is collected in the test tube.

The camera which is placed below the test tubes captures an image of these test tubes and sends it to computer to for image processing.

6.5 CFD Analysis



a) b)

Fig 6.18 a) velocity and b) Pressure CFD

6.5.1 Results and Conclusion

As we can see from above pictures of analysis fig a) shows the complete analysis of velocity in Object Y in Y-axis. Asper the calculation the max velocity is 2.651 which is safer and sustainable. b) fig b) shows the exact variation of pressure difference during the process, the max pressure is 932.8 N/mm^2 drops the pressure up to 893.67 while operation, object y is sustainable during this working pressure which is safer.

6.5.2 Cost of manufacturing:

Components	Material	Rate	Quantity	Cost
Blood Antigens		260/-	1	260/-
Administrative Blood Kit		200/-	1	200/-
Three Way	Plastic	100/-	3	300/-
Syringe	Plastic	10/-	6	60/-
EDTA Dilution		500/-	1	500/-
Rack and Pinion	Fiber	150/-	2	300/-
Test Tube	Glass	80/-	3	240/-
Stepper Motor		200/-	1	200/-
Arduno UNO		750/-	1	750/-
Glass strip	Glass	50/-	1	50/-
Plywood	Wood	500/-	1	500/-
Camera		1200/-	1	1200/-
			Total	4560/-

Table 2.7 Cost of Manufacturing Prototype

CHAPTER 7

BLOOD IMAGE PROCESSING

7.1 Image Acquisition process

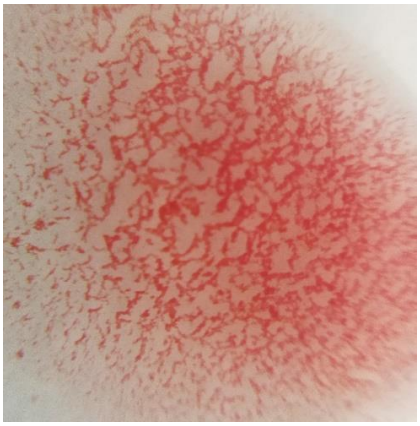


Fig. 7.1 Original Blood Smear Microscopic Image

This image depicts the antibodies formed in the mixture of blood and antigen, that is captured by the camera operated with the help of Raspberry pie.

Image pre-processing as follows

7.1.1 Gray Scale Image

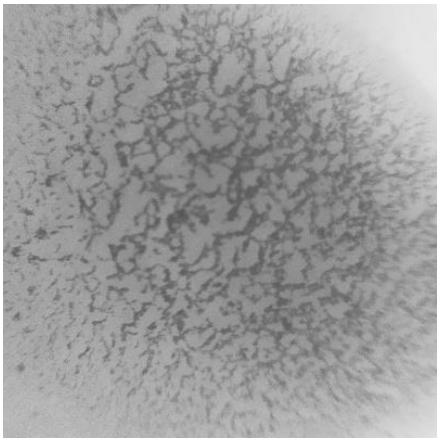


Fig. 7.2 Gray Scale Image

In image processing process, grey scale image is a preliminary stage to perform on a raw picture, to work on single channel.

7.1.2 Thresholding Processes

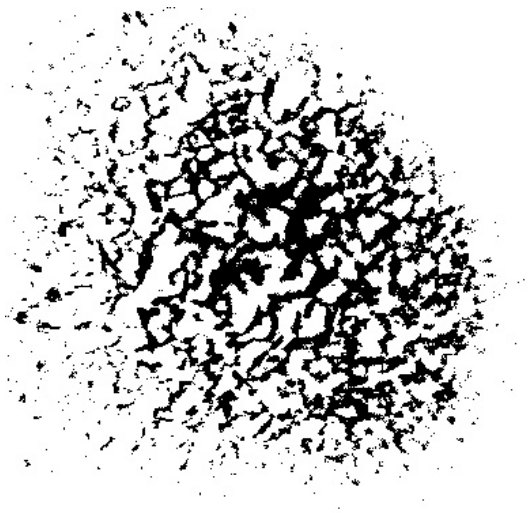


Fig. 7.4 Threshold

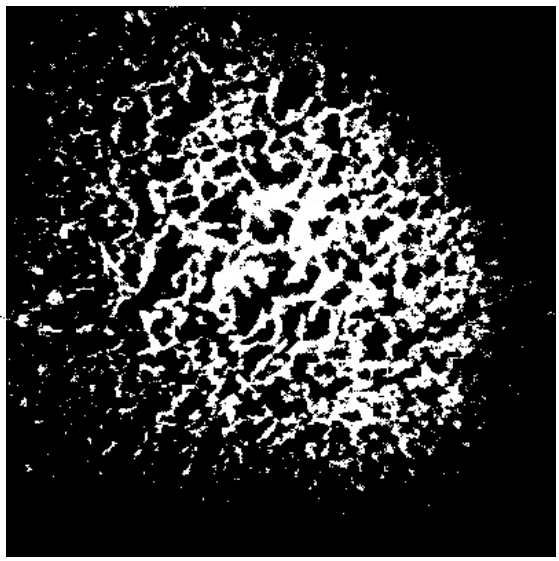


Fig. 7.5 Inverse Threshold

Thresholding Process is used to extract selected data from the image by suing pixel values also used to manage pixels and dived all the values in two parts, with the help of thresholding process we can delete the background of the image vice versa.

Thresholding operation in image processing is used to create binary images. The Gray scale samples are clustered into two parts as background and object. In this case, multilevel thresholding is performed using Otsu's method. More than one threshold is determined for a given image and segmentation is done creating certain regions. One background with many objects is the result of this multilevel thresholding. It is a clustering-based image thresholding. Figure 5 shows the result of multilevel thresholding using Otsu's method.

7.1.3 Adaptive Thresholding

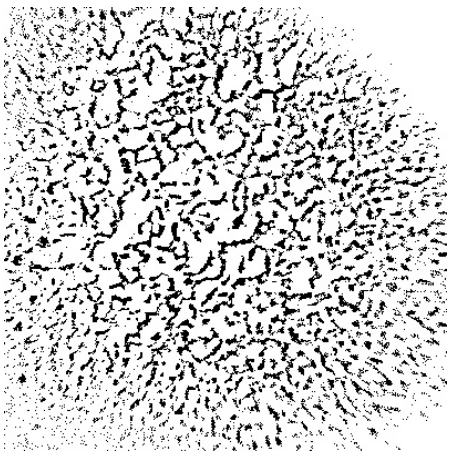


Fig. 7.6 Adaptive Thresholding

Adaptive Thresholding Process used to find out different types of low luminous pixels on small regions of the image.

7.1.4 Morphological Process

Morphological Transformation are some simple operations based on a image shape, it is normally performed on a binary image

Morphology is a tool of extracting image components that are useful in the representation and description of region shape, such as boundaries, skeletons, and the convex hull. In morphological operation, there are two fundamental operations such as dilation and erosion, in terms of the union of an image with translated shape called a structuring element. This is a fundamental step in extracting objects from an image for subsequent analysis.

1. Dilation

Dilation is the process that grows or thickens the objects in an image and is known as structuring element. Graphically, structuring elements can be represented either by a matrix of 0s and 1s or as a set of foreground pixels. The dilation of A by B is set considering all the structuring element origin locations where the reflected and translated B overlaps at least one element. It is a convention in image processing that the first operand of AB be

the image and the second operand is the structuring element, which usually is much smaller than the image

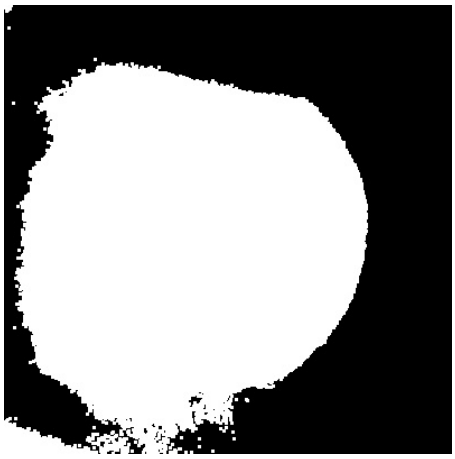


Fig 7.7 Dilation

2. Erosion

Erosion shrinks or thins objects in binary image. The erosion of A by B is the set of all points z. Here, erosion of A by B is the set of all structuring element origin locations where no part of B overlaps the background of A. In image processing applications, dilation and erosion are used most often in various combinations. An image will undergo a series of dilations and erosions using the same, or sometimes different, structuring elements. The most important combinations of dilation and erosion are opening and closing

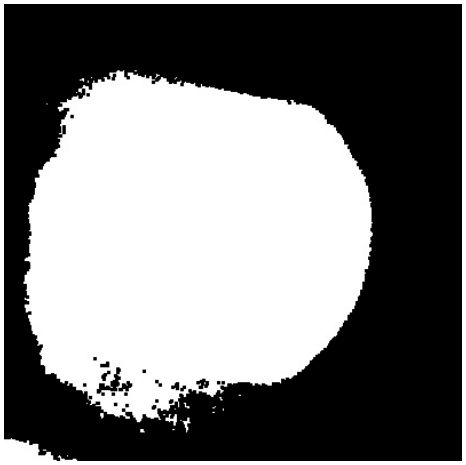


Fig 7.8 Erosion

7.1.5 Image Filtering and Edge Detecting Process

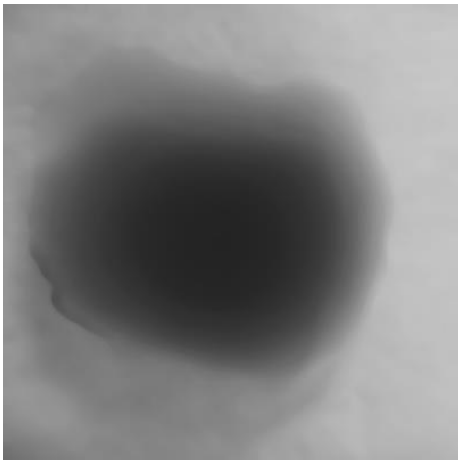


Fig 7.9 Median Filter Process

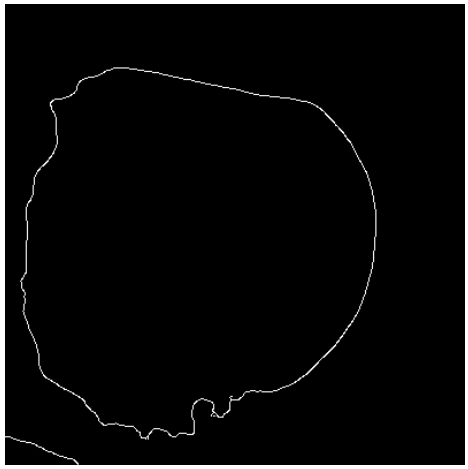


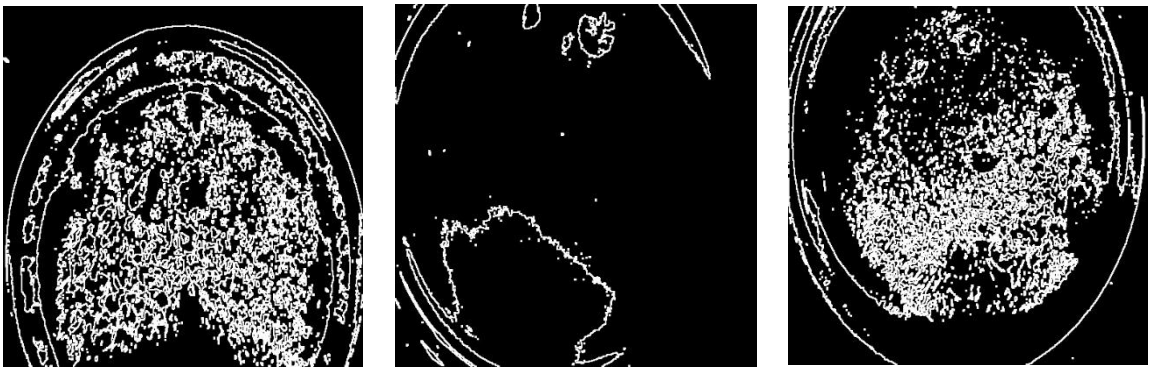
Fig. 7.10 Canny Edge Detection on Median Filtered Image

The output from the pre-processing operation is further treated in threshold operation processing. Thresholding operation in image processing is used to create binary images. The grey scale samples are clustered into two parts as background and object. It is a clustering-based image thresholding. Each fig shows the edge detection using ‘Canny Edge Detection’ technique.

7.2 Result

The image from the threshold operation is compared with the image preset in the Raspberry pie and evaluated for the blood group of the person. Hence, detection of blood is completed after the evaluation of the image.

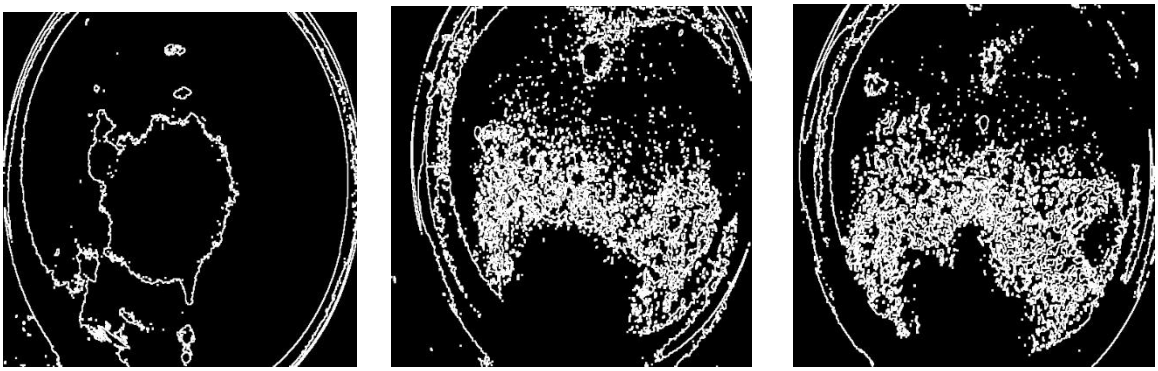
a) Result for A+



a) b) c)

Fig 7.11 Result of A Positive a) Antigen A b) Antigen B and c) Antigen D respectively

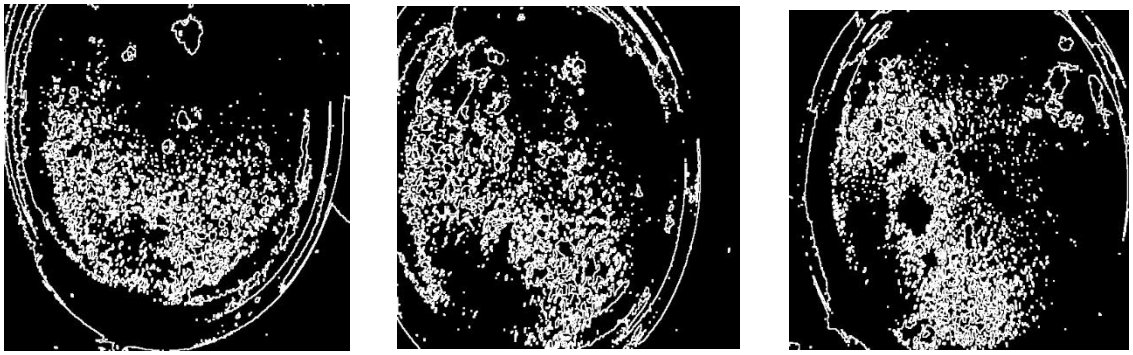
b) Result for B+



a) b) c)

Fig 7.12 Result of B Positive a) Antigen A b) Antigen B and c) Antigen D respectively

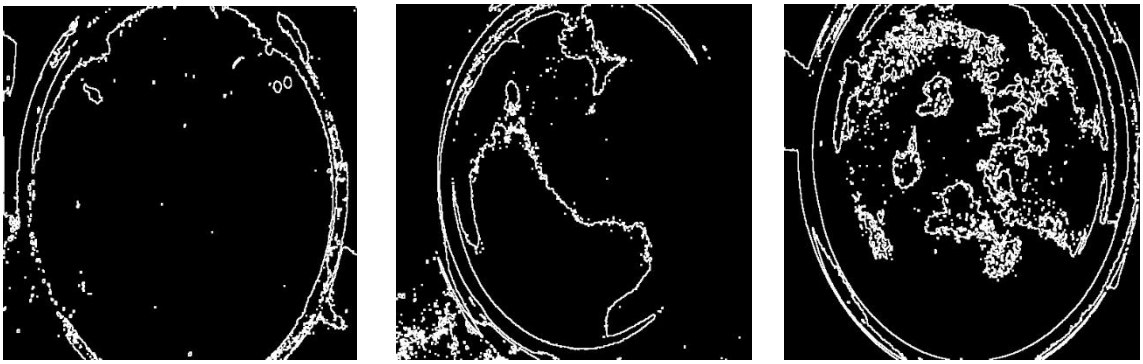
c) Result for AB+



a) b) c)

Fig 7.13 Result of AB Positive a) Antigen A b) Antigen B and c) Antigen D respectively

d) Result for O+



a) b) c)

Fig 7.14 Result of O Positive a) Antigen A b) Antigen B and c) Antigen D respectively

Code –

Image Processing Programme

```
import cv2
import numpy as np

cam = cv2.VideoCapture(1)

cv2.namedWindow("Capture Imgae")

img_counter = 0

while True:
    ret,frame = cam.read()

    if not ret :
        print("failed to capture")
        break

    cv2.imshow("frames",frame)

    k = cv2.waitKey(1)

   # if k == ord("q"):
     #   break

    if k == ord("c"):
```

```
        cv2.imwrite('E:\\Project\\img_name_{}.png',frame)

        img_counter+=1


        break
cam.release()


img = cv2.imread("E:\\Project\\img_name_{}.png",0)
img = cv2.resize(img,(500,500))

cv2.imshow("orignal",img)

_,th1 = cv2.threshold(img,50,255,cv2.THRESH_BINARY)
_,th2 = cv2.threshold(img,50,255,cv2.THRESH_BINARY_INV)
_,th3 = cv2.threshold(img,127,255,cv2.THRESH_TRUNC)
_,th4 = cv2.threshold(img,100,255,cv2.THRESH_TOZERO)


cv2.imshow("1-Thresh Binary",th1)
cv2.imshow("2-Thresh Binary Inv",th2)
cv2.imshow("3-Thresh Trunc",th3)
cv2.imshow("4-Thresh Towards zero",th4)

_,th5 = cv2.threshold(img,127,255,cv2.THRESH_BINARY)
```

```
th6 =
cv2.adaptiveThreshold(img,255,cv2.ADAPTIVE_THRESH_MEAN_C,cv2.THRESH_BINARY,11,9)

th7 =
cv2.adaptiveThreshold(img,255,cv2.ADAPTIVE_THRESH_GAUSSIAN_C,cv2.THRESH_BINARY,11,1
1)



cv2.imshow("original",img)

cv2.imshow("Thresh Binary",th1)

cv2.imshow("Adaptive 1",th2)

cv2.imshow("Adaptive 2",th3)

_,mask = cv2.threshold(img,165,255,cv2.THRESH_BINARY_INV)

kernel  = np.ones((3,3),np.uint8)

e = cv2.erode(mask,kernel)

cv2.imshow("Orignal",img)

cv2.imshow("mask",mask)

#cv2.imshow("kernel",kernel)

cv2.imshow("Erosion",e)

kernel = np.ones((3,3),np.uint8)

d = cv2.dilate(mask,kernel)

cv2.imshow("Dilation",d)

_,mask = cv2.threshold(img,165,255,cv2.THRESH_BINARY_INV)

kernel  = np.ones((3,3),np.uint8)
```

```
o = cv2.morphologyEx(mask,cv2.MORPH_OPEN,kernel)



cv2.imshow("Orignal",img)
cv2.imshow("mask",mask)
#cv2.imshow("kernel",kernel)
cv2.imshow("Opening",o)



kernel  = np.ones((3,3),np.uint8)
c = cv2.morphologyEx(mask,cv2.MORPH_CLOSE,kernel)

cv2.imshow("Closing",c)

x1 = cv2.morphologyEx(mask,cv2.MORPH_TOPHAT,kernel) #Dif/B mask and opening
x2 = cv2.morphologyEx(mask,cv2.MORPH_GRADIENT,kernel) #Dif/B Dilation And Erosion
x3 = cv2.morphologyEx(mask,cv2.MORPH_BLACKHAT,kernel)

cv2.imshow("x1",x1)
cv2.imshow("x2",x2)
cv2.imshow("x3",x3)

cv2.imwrite("E:\\Project\\Micro photos\\original.JPG",img)
cv2.imwrite("E:\\Project\\Micro photos\\th1.JPG",th1)
cv2.imwrite("E:\\Project\\Micro photos\\th2.JPG",th2)
cv2.imwrite("E:\\Project\\Micro photos\\th3.JPG",th3)
cv2.imwrite("E:\\Project\\Micro photos\\th4.JPG",th4)
```

```
cv2.imwrite("E:\\Project\\Micro photos\\th5.JPG",th5)
cv2.imwrite("E:\\Project\\Micro photos\\Adp th6.JPG",th6)
cv2.imwrite("E:\\Project\\Micro photos\\Adp th7.JPG",th7)
cv2.imwrite("E:\\Project\\Micro photos\\Mask.JPG",mask)
cv2.imwrite("E:\\Project\\Micro photos\\Erosion.JPG",e)
cv2.imwrite("E:\\Project\\Micro photos\\Dilation.JPG",d)
cv2.imwrite("E:\\Project\\Micro photos\\Morphology Tophat.JPG",x1)
cv2.imwrite("E:\\Project\\Micro photos\\Morphology Gradient.JPG",x2)
cv2.imwrite("E:\\Project\\Micro photos\\Morphology Blackhat.JPG",x3)

cv2.waitKey()
cv2.destroyAllWindows()
```

Stepper Motor Programme

```
#include <Stepper.h>

const int stepsPerRevolution = 200;  // change this to fit the number of steps per revolution
// for your motor

// initialize the stepper library on pins 8 through 11:
Stepper myStepper(stepsPerRevolution, 8, 10, 9, 11);

void setup() {
  // set the speed at 60 rpm:
  myStepper.setSpeed(60);
  // initialize the serial port:
  Serial.begin(9600);
}

void loop() {
  // step one revolution  in one direction:
  Serial.println("clockwise");
  myStepper.step(700);
  delay(500);

  // step one revolution in the other direction:
  Serial.println("counterclockwise");
  myStepper.step(-700);
  delay(500);
```

CHAPTER 4

CONCLUSION AND FUTURE SCOPE

The purpose of this project was to reduce the time required to determine the blood group of the person also to reduce the production of biowaste compared to the methods which were earlier used to determine the blood groups.

The conventional methods mentioned in the report requires time requires time and energy to perform the procedure. Also, it was found that these methods generate bio-waste. With the help of the experiment and the prototype it is validated that these problems can be solved with the implementation of concepts such as microfluidics and image processing.

The prototype also validates the nature of blood in under pressure, as the pressure of blood was increased during the flow of blood through the microchannel the time required for the mixing of blood with antigen solution was reduced. Hence, fastening the process of Agglutination of Red Blood cells. It was found that to determine the blood group 6 seconds are required which in contrast to the earlier methods is much lesser.

With this result, which we were able to accomplish the objectives of this project by successfully solving the problem and making a device which will help the physicians to determine the blood group.

References:

1. David Nobes, Md Ashkar Ibney Rashid, Shadi Ansari, Prashant Waghmare, "Capillary Filling of Non-Newtinian Fluids", June 2015, researchgate.com, Paper Id-133, publication 3237630870.
2. Franz L. Dickert, Adnan Mujahid, Vittorio M. N. Passaro, "Blood Group Typing: From Classocal Strategies to the Applications of Synthetic Antibodies Genrated by Molecular Imprinting", 31st Dec 2015, National Center for Biotechnology Information, PMCID: PMC4732084, PMID – 26729127.
3. Beckman Coulter PK systems, "Blood Group Reagents", May 2020, pp 1-10.
4. Geoff Daniels, Imelda Bromilow, "Essential Guidee to Blood Groups", 2007, Blackwell Publishing, pp-1-109.
5. G. Mohiuddin Mala, Dongqing Lit and J. D. Dale, "Heat transfer and fluid flow in microchannels", 1996, International. Journal of Hear Mass Transfer, Vol. 40 (13), pp-3079-3088.
6. D. Liu and S. V. Garimella, "Investigation of Liquid Flow in Microchannels", 2004, AIAA Journal of Thermophysics and Heat Transfer, Vol. 18(1), pp. 65-72.
7. David W. Inglis, John A. Davis, Thomas J. Zieziulewicz, David A. Lawrence, Robert H. Austin and James C. Sturm, "Determining Blood Cell Size Using Microfluidic Hydrodynamics" , 2007, Journal of Immunological Methods, Volume 329 (2008), pp 151–156.
8. Bernhard Sebastian and Petra S. Dittrich, "Microfluidics to Mimic Blood Flow in Health and Disease", 2018, Volume 50, pp 483-504.
9. Melinda A. Lake, Cody E. Narciso, Kyle R. Cowdrick, Thomas J. Storey, Siyuan Zhang, Jeremiah J. Zartman, and David J. Hoelzle, "Microfluidic device design, fabrication, and testing protocols", 2015, Protocol Exchange.
10. Adelina-Gabriela Niculescu, Cristina Chircov, Alexandra Catalina Bîrca and Alexandru Mihai Grumezescu, "Fabrication and Applications of Microfluidic

Devices: A Review", 2021, International Journal of Molecular Sciences, Volume 22 (4)

11. Ankita Dalvi, Hanu Kumar Pulipaka, "Determination of Blood group using Image processing" March 2018, ,Internationa journal of Scientific and Engineerig and Research Volume 9 Issue 3.
12. Mrs.G.Sangeetha Lakshmi, Ms.M.Jayashree., "Automated Blood Group Recognition System Using Image Processing" April 2019, International Research Journal of Engineering and Technology, Volume 06 Issue 04.
13. Anushka Gulati, Rutu Patel, Vishal Ajith Mathew, M. Jagannath, "ABO Blood Group Determination System Using Texture Feature and SVM Classifier", 2020, Journal of Critical Reviews, Volume 07 Issue 05.