Design and Construction of a Low-Cost Hemoglobin Electrophoresis (Genotype) Machine for the Diagnosis of Inherited Genotype Disorder

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Abstract. Designing and constructing a low-cost haemoglobin electrophoresis (genotype) machine using cellulose acetate paper is an exciting project that underscores using locally sourced materials and resources to achieve its aims. The technique of haemoglobin electrophoresis is adequately harnessed to detect and evaluate various types of haemoglobin disorder, which include sickle anaemia and many other abnormal genotypes. The cost of acquisition and maintenance of the equipment is made available at a comparatively reduced rate by using materials and components that are remotely accessed to construct the haemoglobin electrophoresis machine, which is efficient and cost-effective. The design and construction team incorporated a power supply unit, buffer solution (tris), cellulose acetate paper, filter paper, electrodes (anode and cathode), switch, cable, capacitor, fuse, and other necessary components. The successfully constructed genotype machine was validated for effective and efficient diagnostic purposes for detecting different types of haemoglobin genotypes by testing it using several specimens of known haemoglobin genotypes of different types. The researchers compared the results from this testing process with those obtained from a standardised haemoglobin genotype machine. The test result obtained from this verification and validation process showcases that the low-cost haemoglobin electrophoresis ma-chine using cellulose acetate paper can effectively separate, distinguish and identify different haemoglobin variants such as HBAA, HBAS, HBAC, HBCC, HBSS, HBSC, HBF, HBAC, HBAF with few limitations. The drawbacks include the inability to distinguish other haemoglobin genotype variants that co-migrate with HBAA, HBAS, HBAC, HBCC, HBSS, HBSC, HBF, HBAC, and HBAF when exposed to the electric field in the electrophoretic chamber. Examples of such haemoglobin genotypes include HB E, HB D, HB O, and HB G, with many other haemoglobin variants having exact migration patterns on the cellulose acetate paper when subjected to the electric field. However, this limitation can be overlooked as these haemoglobin variants are not peculiar to people in this part of the world (Nigeria) where the project has been carried out. Hence, the design and construction of this haemoglobin electrophoresis machine will give people from low-resource areas easy access to carry out the test and enhance prompt detection and management of hemoglobinopathies.

Keywords: Hemoglobin; Electrophoresis; Cellulose Acetate Paper; Hemoglobin Electrophores; Hemoglobinopathy; Validation; Hemoglobin-electrophoresis; Genotype.

INTRODUCTION

Electrophoresis is a crucial test among several methods used to determine haemoglobin variants, categorised as a chromatography technique [1]. This methodology deploys an electrically charged field to enhance the migration of molecules.

Hemoglobin variants are often determined using the chromatography methodology known as electrophoresis [1]. In this technique, the electric field enhances the movement of electrically charged molecules. The first haemoglobin variant (Hb-S) was described using the electrophoresis technique in 1949.

The most popular monogenic abnormality across the globe is hemoglobinopathies, a genetic disorder related to the production of haemoglobin [2]. The DNA variants residing in the globin genes, which code for the chains of globin at the tetrameric haemoglobin level, are responsible for the genetic mutation of these disorders [3, 4]. The aftermath result of these DNA variants often truncates the production of α- or β-globin or leads to changes in the structure of haemoglobin, thereby leading to disorders like sickle cell disease, erythrocytosis or polycythemia and hemolytic anaemia. Haemoglobin gene variants are being carried by almost 7% of the earth's population [4]. The result of haemoglobin variant mutations, which are inherited-homozygous (HbSS), leads to sickle cell disease (SCD) or is shared with different globin gene mutations [1, 4]. Some of the clinical symptoms of sickle cell anaemia are chronic hemolytic anaemia, organ dysfunction, acute painful episodes, stroke and early mortality [5].

A diagnostic technique determining the different types of haemoglobin inherent in a patient's blood sample is haemoglobin electrophoresis. This type of test helps to identify haemoglobin diseases, which are genetic mutations that affect the production and structure of haemoglobin. Examples of haemoglobin disorders include sickle cell anaemia, thalassemia, and haemoglobin C disease.

According to the American Society of Hematology (ASH), haemo-globin electrophoresis is harnessed to differentiate haemoglobin variants based on their electric charges [1, 6]. ASH established that this test is generally used to determine sickle cell disease, traits, and thalassemia.

The World Health Organization (WHO) also recommended that haemoglobin electrophoresis is an essential diagnostic tool for hemoglobinopathies [2]. WHO also noted that this technique is valuable for the detection of carriers of abnormal haemoglobin and for the diagnosis of affected individuals.

Research Problem. About Baysal, abnormal types of haemoglobin can be identified using a vital laboratory chromatography technique known as haemoglobin electrophoresis, and there is a need for easily accessed haemoglobin electrophoresis worldwide based on usage and affordability [7]. Keren underscored the importance of instrumentation to haemoglobin electrophoresis, while Shih emphasised the step-by-step algorithm approach in determining hemoglobinopathy [8, 9]. Therefore, this study identifies the technical requirements for constructing a cost-effective and user-friendly haemoglobin electrophoresis machine that can be used in general laboratory settings worldwide.

Many studies have been conducted to determine the technicality and design involved in constructing an affordable and user-friendly haemoglobin electrophoresis machine. For example, Keren proposed that a genotype machine should be able to carry out several forms of electrophoretic tests using cellulose acetate strips and agarose gel electrophoresis [8]. More importantly, several variants of haemoglobin, such as HbA, HbF, HbC, and HbS, should be detected using this machine.

The sensitivity and specificity level of the electrophoretic machine should be high enough to detect haemoglobin variants [9]. In addition, the machine should be user-friendly and easy to operate, even by personnel with limited skills in haematological practice. The machine should be well coupled to be easily transported to remote locations.

The importance of proper sample preparation and handling cannot be over-emphasised while determining haemoglobin genotype using haemoglobin electrophoresis [7]. Therefore, the machine is expected to have an efficient sample preparation system that can carry out many samples concurrently.

Engineers and designers should thoroughly and carefully consider the technical know-how and design requirements when constructing a low-cost and user-friendly haemoglobin electrophoresis machine. Some of these requirements in-

volve the capacity to determine various haemoglobin (genotype) variants, a high level of sensitivity and specificity, user-friendliness, affordability, and a compact and efficient sample preparation system.

Aim and Objectives of the Study. This project aims to design and construct a low-cost haemoglobin electrophoresis (genotype) machine to diagnose inherited genotype disorder.

The objectives of this project are to:

1) Design a machine that can perform genotype tests.

2) Construct an electrophoresis machine that can detect various haemoglobin gene variants, including normal haemoglobin (HbA), haemoglobin S (HbS), haemoglobin C (HbC), and other haemoglobin variants.

3) Ensure the machine has high sensitivity and specificity to determine haemoglobin gene variants.

4) Construct an electrophoretic machine that is user-friendly and has little expertise.

5) Produce a low-cost machine that is affordable by healthcare facilities with limited resource settings.

6) Be able to process multiple samples simultaneously.

7) Ensure its accuracy and reliability in detecting haemoglobin variants in patient samples.

The following highlights the significance of the constructed haemoglobin electrophoresis machine:

1) *Enhance the Management and Diagnosis of Abnormal Genotypes:* Haemoglobin electrophoresis is essential for diagnosing and identifying different haemoglobin genotypes in human blood samples. A well-constructed haemoglobin electrophoresis machine that is reliable and precise can aid in the diagnosis and control of various haemoglobin disorders; examples of such include sickle cell disease, thalassemia and haemoglobin C disease.

2) *Heightened the Rate of Access to the Test:* Most often, the tests carried out for the detection of genotypes are done in specialised laboratory settings, which may hinder easy accessibility to patients in low-income or resource-limited locations. Therefore, with the construction of less expensive and compact haemoglobin electrophoresis, people will have more access to determine their haemoglobin genotype, especially in locations where specialised machines and expertise may be limited.

3) *Enhanced Patient Improvement:* the quality of life and patient outcome are adequately achieved by timely diagnosis of hemoglobinopathies. Reducing the price and cost of tests will help to enhance the early detection and control of abnormal haemoglobin.

4) *Test Standardisation:* Constructing a well-standardised haemoglobin genotype machine will provide precise and accurate test results across most resource-limited diagnostic centres.

5) *Research Opportunities:* this project will allow researchers to conduct more detailed studies on hemoglobinopathies and their variants.

This project focuses on designing and constructing a haemoglobin electrophoresis machine which is reliable and precise for identifying different types of haemoglobin variants in human blood samples. The machine uses cellulose acetate paper as a medium through which the blood smear migrates when placed in an electric field. The haemoglobin electrophoresis machine can detect different haemoglobin variants, which involve normal haemoglobin (HbA), haemoglobin S (HbS), haemoglobin C (HbC), and other haemoglobin variants. The machine should be user-friendly, cost-effective, and well-coupled with an efficient sample preparation structure.

Limitations

1) The problem encountered in detecting all forms of haemoglobin variants and their complexity has been underscored in many studies [10, 11]

2) In a review by Mast, the cost of producing a haemoglobin electrophoresis machine was highlighted, emphasising the need for accessible and affordable diagnostic tools for hemoglobinopathies [12].

3) Several studies, including Huisman and a review by Enevold, showcased the technical skills required for constructing a haemoglobin electrophoresis machine [13, 14].

4) Several sources, including a review by Higgins and a study by Meissner, have reinstated the significance of validation and testing to ensure the reliability and accuracy of the haemoglobin electrophoresis machine [15, 16].

The basis of this construction, "haemoglobin electrophoresis machine using cellulose acetate strip", is justified by its immense contribution to managing and identifying normal and abnormal haemoglobin caused by a genetic mutation that truncates haemoglobin synthesis [12]. A laboratory technique that precisely identifies different types of haemoglobin molecules based on their electric charge and migration pattern is haemoglobin electrophoresis, which is paramount for detecting hemoglobinopathies such as sickle cell anaemia and thalassemia [14]. Healthcare professionals can improve accuracy and timely diagnosis using a haemoglobin electrophoresis machine, leading to prompt medical attention and better patient management [12]. Genetic screening and counselling can also be conducted using haemoglobin electrophoresis to help identify carriers of hemoglobinopathies and reduce the risk of transmission to future lineage [14]. Hence, the tendency to enhance the management and diagnosis of hemoglobinopathies justifies constructing a haemoglobin electrophoresis machine, which will help patients improve and lower the stress of these genetic disorders on individuals and the community.

The primary justification for this project is to heighten easy access to accurate diagnosis and management of abnormal haemoglobin genotype in a setting with limited or low resources. In most developing countries, an increase in the cost of diagnosis for hemoglobinopathies can hinder timely and effective care. As a result, developing an affordable haemoglobin Electrophoresis machine will help health providers and researchers in this clime to quickly screen for hemoglobinopathies and monitor the patient's treatment. Moreover, this machine can be an essential tool for researching the prevalence and distribution of haemoglobin disorder across different populations, which can aid decision-making, policies and public health interventions. More accessibility to technology will, in turn, recreate local capability for diagnosis research and, more importantly, enhance healthcare services for people and regions affected by haemoglobin genotype disorder.

Definition of Key Terms

Haemoglobin: the complex amino acid found in blood cells responsible for transporting oxygen from the lungs to the rest of the body tissue is known as haemoglobin [11].

Electrophoresis: Electrophoresis is the laboratory technique that separates molecules according to their charge and size [13].

Haemoglobin Electrophoresis: A technique that differentiates and identifies various types of haemoglobin concerning their electrical charge is called haemoglobin electrophoresis [12].

Hemoglobinopathy: A genetic abnormality that affects the structure or synthesis of haemoglobin, including sickle cell anaemia and thalassemia, is called hemoglobinopathies [14].

Validation: The process of testing and verifying that a haemoglobin electrophoresis machine operates as intended and produces accurate results is called validation [15].

Literature review

A laboratory technique used for identifying several types of haemoglobin and diagnosing haemoglobin disorders, which are genetic abnormalities that affect the structure and synthesis of haemoglobin, is called haemoglobin electrophoresis. Researchers generally harness this technique for diagnosing abnormal haemoglobin, and recent studies have underscored its relevance in the accurate and prompt diagnosis of these abnormalities [17, 18].

It is based on the electrical charge and size of haemoglobin molecules using an electric field and a support medium, such as cellulose acetate or agarose gel, that haemoglobin electrophoresis deploys to distinguish the type of haemoglobin genotype. This methodology gives unique bands that can be seen using a staining solution.

The significance of haemoglobin electrophoresis when diagnosing haemoglobin genotype disorder has been reiterated in recent studies, majorly in high-prevalent regions such as the Middle East and Africa. For example, El-Beshlawy used haemoglobin electrophoresis to diagnose hemoglobinopathies in many Egyptian patients [17]. The authors reported that the technique was susceptible and specific, so they recommended it as a valuable tool for diagnosing these abnormalities.

Haemoglobin electrophoresis is also used to detect abnormal haemoglobin in Iranian patients. The researcher discovered that the method efficiently identified the types of haemoglobin disorders, and essential information was provided to control and treat these abnormalities [18]. Notably, haemoglobin electrophoresis is an invalua-

ble tool for diagnosing haemoglobin genotype disorder. Recent research has underscored the significance of this technique in precise and prompt diagnosis of hemoglobinopathies, especially in regions where this is highly prevalent. This methodology is also pivotal to knowing the genetic basis of these disorders and helping control the necessary treatment options [1, 2].

Thalassemia and sickle cell disease are determined at an alkaline pH of 8.4 using diagnostic tools known as alkaline electrophoresis. The hemolysis of the red cell is initially prepared with the aid of hemolysate to lyse the red cells. A diagnostic technique such as alkaline electrophoresis determines thalassemia and sickle cell disease at pH 8.4. Firstly, the blood cells are lysed with hemolysate, then well positioned on a cellulose acetate strip and subjected to run through a buffer at a stable voltage in an electrophoresis chamber [19]. Therefore, the types of haemoglobin with net charges that are not the same are severed into bands of different types, depending on their mobility. HbS and HbC can be differentiated by haemoglobin electrophoresis, a clinically significant variant. It should, however, be noted that variants of haemoglobin with the exact electrical charges that also produce the same pattern of movement cannot be differentiated by haemoglobin electrophoresis. Examples of these are HbD and HbG, which have the exact migration pattern with HbS, HbE, and HbO-Arab, with the same pattern of movement as the molecules of HbC [20, 21].

Moreover, a significant amount of haemoglobin F that is present in newborns can be affected by alkaline electrophoresis, which may overshadow the little haemoglobin band. Hence, more care should be ensured to determine the HbS reliably. Also, less significant bands like HbA2, HbH, and Hb Bart's can be misplaced. As a result, more robust techniques can be deployed as a diagnostic tool to address these drawbacks [22].

Techniques and Assays to Diagnose and Monitor Genotype Disorder. The determination and control of haemoglobin disorder with the inclusion of sickle cell traits are undertaken by myriads of techniques. These are categorised into two main divisions: 1) Recently used methods for detecting sickle cell disease (SCD); 2) the use of point of care (POC) for detecting SCD and various innovative methodologies are still under review or in the research stage [22-25].

The technique often used to diagnose abnormal haemoglobin genotype is cellulose acetate electrophoresis. [19, 26]. Using an electric field and cellulose acetate paper as a support medium, this technique separates protein molecules according to their charge and size. To determine genotype disorders using cellulose acetate paper, the first step is to harvest and purify the protein of interest from the patient's specimen [26]. The lysed and purified protein is mixed with a buffer solution and carefully loaded onto a cellulose acetate strip using various methods. After positioning the cellulose acetate paper in the electrophoretic chamber, an electric field is applied via the power source. The mobility of the emulsified proteins through the strip is based on the charge and size of the haemoglobin, which gives distinct bands that can be seen and identified using the staining solution. The array and distinction of these bands are compared to a reference standard to determine the presence of any mutation or genotype disorder [1, 26].

Cellulose acetate electrophoresis is the standard method to detect various haemoglobin genotype disorders, including hemoglobinopathies, alpha-1 antitrypsin deficiency, and cystic fibrosis [19, 26]. Al-Mosawi highlighted in his research the critical use of cellulose acetate electrophoresis to diagnose haemoglobin disease in Iraqi patients. He reported that the technique was exact and dependable in identifying the types of hemoglobinopathies [26].

Kumar also used cellulose acetate electrophoresis in his research to diagnose alpha-1 antitrypsin deficiency in Indian patients. He deduced that the technique was sensitive and specific. Therefore, he recommended it as a valuable tool for diagnosing this abnormality [19]. Hence, the use of cellulose acetate electrophoresis is a reliable technique for the diagnosis of haemoglobin disorders [19, 26] as this technique is relatively user's friendly and cost-effective and can be made available in most clinical laboratories

Sickle Cell Disease and Other Genotypes Detected by Hemoglobin Electrophoresis. Sickle cell disease, thalassemia, and structural haemoglobin variants, such as haemoglobin C and E, can be described as the different types of hemoglobinopathies diagnosed by haemoglobin electrophoresis [27]. Singh underscored the significance of accurately diagnosing and classifying haemoglobin disorder for appropriate clinical management.

One of the major causes of gene abnormalities with significant mortality and morbidity across the world is hemoglobinopathies. Hence, adequate usage of readily available screening and testing methods is essential for its detection and control [28].

Moreover, in a recent publication, Manzoor evaluated using haemoglobin electrophoresis to diagnose hemoglobinopathies in the Pakistani population. An increase in the prevalence of beta-thalassemia and haemoglobin E disease was identified by haemoglobin electrophoresis [29].

All these reviews showcase the progressive relevance of haemoglobin electrophoresis as an instrumental diagnostic technique for diverse haemoglobin disorders, emphasising the significance of precise diagnosis and classification for proper clinical control.

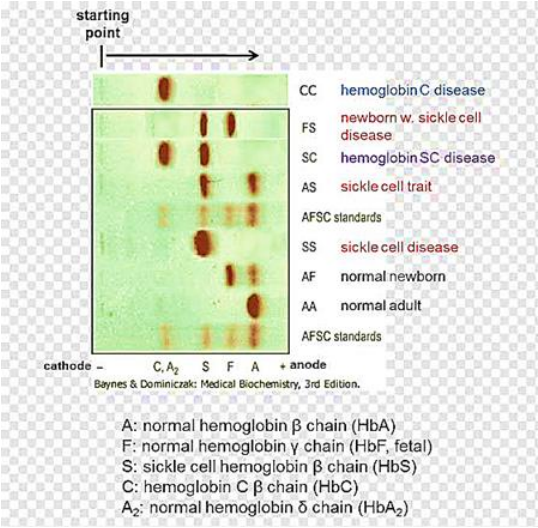

Figure 1 – Genotype Test Result [30]

Explanation of the Different Genotype Test Results. The molecules of the haemoglobin genotype can be classified into several types: Hb A, HbS, HbF, and HbC; all these are present in the haemoglobin, which acts as a transportation medium of oxygen within the body system [31].

Haemoglobin A (HbA) consists of two alpha and two beta globin protein chains, which comprise several individuals' average adult haemoglobin molecules. Hb S, or Hemoglobin S, results from the mutation of Hb A in the beta-globin gene, which is linked to sickle cell disease. Also, in the developing fetus and newborn, fetal haemoglobin is present, which consists of two alpha and two gamma globin protein chains. This Hb F is produced in varying amounts in adults, with few people still expressing it into adulthood. Another form of the average adult haemoglobin A mutation is the Hemoglobin C (Hb C), the beta-globin gene associated with haemoglobin C disorder [5].

Several other types of genetic mutation that truncate the synthesis and function of haemoglobin are thalassemia, sickle cell disease, and haemoglobin E disease, among many different variants [5]. These abnormalities can lead to diverse symptoms, and there might be a need for frequent medical intervention.

A cluster of genetic abnormalities that affect the synthesis of haemoglobin are called thalassemias. People with these disorders have a lowered amount of haemoglobin or haemoglobin disease, often leading to anaemia, fatigue and several other symptoms. Majorly thalassemia is divided into two main types: alpha thalassemia and beta thalassemia. When we have a mutation in one or more of the alpha-globin genes, it is called Alpha thalassemia, while a mutation that takes place in one or both of the beta-globin genes is called beta thalassemia [5].

One of the Genetic abnormalities affecting the shape of red blood cells is called sickle cell disease. Sickle cell disease occurs in people who possess haemoglobin S, which makes the red blood cells stiff and C-shaped, leading to symptoms such as pain, fatigue, and a high risk of infections. People of African descent are peculiar with sickle cell disease, but it can also be seen among people from other parts of the world [29].

Another genetic disorder affecting the production of haemoglobin is Hemoglobin E disease. Haemoglobin E disease is found in people with a variant form of haemoglobin E; this can lead to mild to severe anaemia, fatigue and several other symptoms. This haemoglobin E disorder is often common in people of Southeast Asian lineage. Also, there is a tendency for it to occur in people from other parts of the world [32].

Divers of symptoms can be the resultant effect of genetic mutation or abnormalities, which have a significant negative impact on haemoglobin and may often need medical intervention, some of which include blood transfusion, iron chelation treatment or bone marrow transplants [29].

Types of Specimens for Hemoglobinopathy Screening and Diagnosis. Sometimes, in the US, healthcare providers require cord blood for screening haemoglobin disorders, but they are now considering using dried blood spots (DBS).

This process involves the collection of DBS from a heel prick and positioned on filter paper, which is a collection of blood placed on a strip for newborn screening [5]. The samples for screening newborn babies should be concisely collected to prevent coagulation and oversaturation. However, many of these samples may not be good enough for screening newborn babies, but they may be suitable for testing for other haemoglobin abnormalities [5].

The screening of children (above one year of age) and adults can be carried out using dried blood spot DBS and liquid whole blood; this is because sometimes, there is a significant alteration in the forms of haemoglobin and its quantities throughout the first year of life, but the haemoglobin types of humans remain the same after a year of age.

Hemoglobin	Structure	Levels at Birth	Levels in Adults	Comments
A	$\alpha_2\beta_2$	20%-25%	97%	Reaches adult levels by 1 year of age
A_2	$\alpha_2\delta_2$	0.5%	2.5%	Elevated in β thalassemia trait
F	$\alpha_2\gamma_2$	75%-80%	< 1%	Reaches adult levels by 1 year of age
HbH	β_4	15%-20% in HbH disease	NA	HbH produces Heinz bodies in the erythrocytes and hemolysis
Hb Bart	γ_4	100% in hydrops fetalis, 15%-25% in HbH disease	NA	Increased in carriers of α thalassemia trait at birth

NA = not applicable.

* Figure courtesy of Deepak Kamat, MD, PhD, FAAP via Healio.com

Figure 2 – Hemoglobin Variant in Children and at Birth

Ethylene Diamine Tetra Acetic Acid (EDTA) is the ideal anticoagulant used to preserve and maintain the integrity of the whole blood sample collection. The finger prick is the site for dried blood spot collection; the last phalanx of the surface of the finger is often used [33]. During transportation and storage, the increase in humidity and degree of temperature should be reduced to retain the integrity of the haemoglobin cells; this is because the level of haemoglobin A and S in dried blood spot specimens can be affected by a raised level of heat and humidity. In haemoglobin disorder testing exercise, the use of blood smear is unreliable and, as such, discouraged because of the possibility of the absence of sickle cells, which may not be in circulation during the time of sample collection [5].

Moreover, blood smears may not detect other haemoglobin variants, and healthcare professionals cannot distinguish between homozygous and heterozygous haemoglobin types using this method. In addition, screening results of abnormal haemoglobin may be affected by transfusion because the blood that has been transfused may contain variants of haemoglobin that are not inherent in the patient's blood. In this transfusion situation, haemoglobin disease screening must be re-carried after four months of transfusion.

Methodologies for Hemoglobinopathy Screening and Diagnosis. US laboratories have used several means to screen for hemoglobinopathy in newborns and adults. Conventionally, electrophoresis is the technique used to detect haemoglobin variants [33].

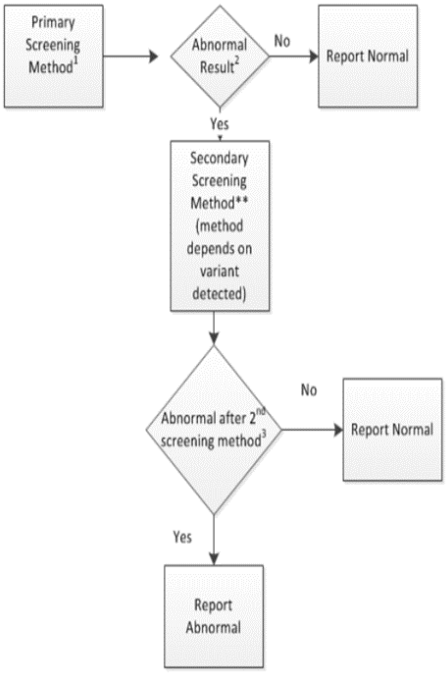

Figure 3 – Basic Hemoglobinopathy Testing Process

The screening of newborn infants has recently come with a more robust technology with higher sensitivity over electrophoresis. Isoelectric focusing (IEF) and High-performance liquid chromatography (HPLC) are also examples of screening techniques. Recently, considering the commencement of the conventional screening of babies, a more advanced innovation with raised outcomes and higher sensitivity over electrophoresis has emerged. Amidst several innovative techniques coming up, the method of choice relies on the function of the laboratory and the policies for conducting tests. The use of molecular

techniques is also taking charge. Laboratories harness it for analysis and diagnostic purposes, and determining hemoglobinopathy's characteristics and clinical outcomes are no exception. Some techniques deployed are conventional methods, automation, and molecular testing [1, 3].

Global Burden of Hemoglobin Disorders. The high impact of hemoglobinopathies on health can be evaluated by examining the death rate of children under five years of age, as many affected children die prematurely and those who survive face severe health challenges. Globally, more than one per cent of couples have the risk for hemoglobinopathies, in which a minimum of one child is affected, and a higher percentage of children affected die untimely. In West Africa, the rate of death in children less than five years is 18.4%; for children given birth to couples that are not at risk for sickle cell disease, the rate is 16.5% in comparison with children given birth by couples not at risk is 40%. More precisely, the family perspective must be considered while assessing the techniques for the health burden orchestrated by genetic disorders [33, 34].

Sickle Cell Disorder. Most people in advanced countries survive adequately into adulthood as they give provisions for neonatal diagnosis and proper patient care [32]. In under-developed countries, many of the children born with sickle cell disorder die without being diagnosed, often as a result of malaria fever; nevertheless, things are taking a gradual change [32]. Recently, approximately 40% of Africa has developed, and accessibility to healthcare services has improved, which has led to heightened survival and an increase in the need for hospital services [35]. The survival rate, quality of life, and reduction in the need for urgent hospital services have been greatly improved through the services orchestrated by the community, which involve providing information, social support, and prophylactic treatments [35]. It should be noted that care for these disorders must be integrated into primary care wherever they are rampant.

In Africa, there is a severe case for the screening of carriers. There are easy and inexpensive techniques for diagnosing adults and infants. Understanding the risk gives room for several options; this includes reducing family size, ensuring infants at risk are adequately diagnosed at birth, and requesting diagnoses before birth.

Timely diagnosis carried out before birth, which is genetically based, is accessible in many African laboratory settings, and it is relatively not expensive, especially if only the sickle cell variant is the focus [35].

In summary, the WHO recommended and confirmed that diagnosis and counselling for abnormal haemoglobin on a genetic basis should be an inherent part of health care in many countries [33]. The estimation has provided a commencement position for the evaluation and service planning of indigenous needs. As a result of haemoglobin disease being a usual point of entrance for genetic focuses into medical care systems, the provision of services, therefore, ought to be designed to enhance community services that are genetically based [36].

Figure 4 – Price of a Typical Hemoglobin Electrophoresis (Genotype) Machine (Price: ₦380,000)

METHOD

Designing and constructing a low-cost genotype machine using cellulose acetate requires careful selection of low-cost components and the choice of cellulose acetate strips. A low-cost genotype machine that can accurately and reproducibly separate and detect DNA fragments is created following the steps below.

Determination of the Requirements: Determining the requirements of the haemoglobin genotype machine is the initial step. Some of these requirements involve the number of specimens to be diagnosed as well as the sensitivity of the machine.

Designing of the Electrophoresis Chamber: The essential component of the machine is the electrophoretic chamber, and the specific requirement is predetermined by its design and func-

tion. Considering this study, the electrophoretic chamber is designed to accommodate cellulose acetate paper, buffer solutions, cathode and anode. The construction of the chamber is done using low-cost materials known as polyvinyl chloride (PVC).

Cellulose Acetate Strips Selection: Commercially available cellulose acetate membranes produced the cellulose acetate. High-quality strips were used to ensure that the separation of haemoglobin bands was accurate and reproducible.

Power Supply Design: off-the-shelf components, which include rectifiers, capacitors, voltage regulators and cables, were used to design and construct a low-cost power supply. The power supply design ensures the voltage and consistent current flow are stable.

Machine Assembling: The machine assembly process is based on the specific design; it is essentially carried out to ensure that all the materials are correctly connected and the machine is adequately calibrated for usage.

Machine Testing: The machine is properly tested to ensure that it is working as expected before being put to use for diagnostic purposes; this is achieved through several tests by using samples of known status to ensure that the machine is precise and can give reproducible results.

The Design of the Electrophoresis Chamber. Designers consider the materials and specifications below for use in designing and constructing the electrophoretic chamber of a low-cost electrophoresis machine. In addition, the chamber is designed to give enough room for materials such as electrodes, filter paper, buffer solution and cellulose acetate paper.

Cellulose Acetate Paper. The commonly used medium for separating haemoglobin cell bands in haemoglobin electrophoresis is cellulose acetate paper. The DNA fragments are separated based on their size and charge by applying an electric field to the cellulose acetate paper. The fragments of DNA migrate via the paper, and their movement is affected by the electric field and the pores in the paper. The fragments are, therefore, allowed to be separated based on their size and charge, through which the sample's genotype can be identified or determined [1, 37].

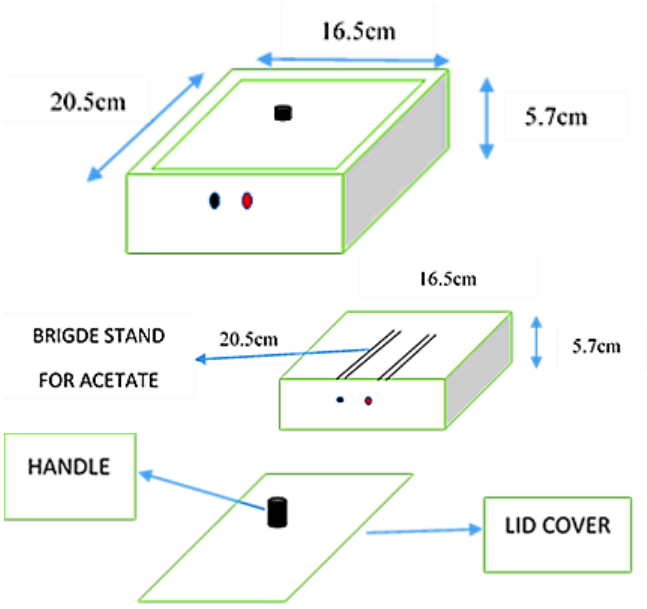

Figure 5 – Design Specification for the Electrophoresis Chamber

The cellulose acetate paper is made to be saturated with 8.4 citrate buffer solution and filtered before use. The cellulose acetate strip is made commercially available on sale.

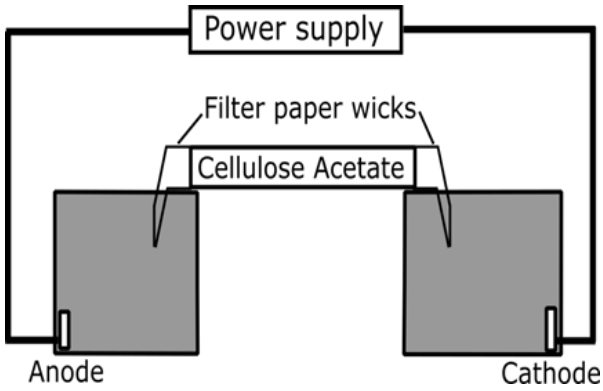

Figure 6 – Cellulose Acetate Paper

Design of the Power Supply. Designers should carefully consider the power requirement, efficiency, voltage stability, size, and brand reputation when designing a power supply unit for a genotype machine. Following these guidelines, the genotype machine can operate reliably and efficiently.

For reliable and efficient performance, the following under-listed parameters are coupled into the power supply unit: 1) Capacitor 400V, 150UF; 2) Rectifier diode; 3) Choke 5W; 4) Power light; 5) AC Meter; 6) Switch; 7) Fuse; 8) Cable.

Function of the 400V, 150UF Capacitor. A 400V, 150UF capacitor in the genotype machine power unit may serve several functions due to the specific design and configuration of the power unit. The functions are:

1) Filtering: the voltage fluctuations or ripple in the power supply are inhibited as the capacitor acts as a filter. With this voltage supply, the various components in the genotype machine are made stable and consistent.

2) Energy storage: electrical energy is stored in a capacitor and used when needed. A 400V, 150Uf capacitor in a genotype machine power unit may provide a quick burst of energy to components that require high current, such as a motor or an amplifier.

3) Power Factor Correction: the system's power factor is improved with a capacitor. Using a genotype machine power unit as a case study, the lower power factor, which may lead to inefficient energy use with heightened operating cost, can be corrected using a capacitor.

4) Regulation of Voltage: the voltage output can be stabilised and prevent voltage spikes or drops that could damage sensitive components in the genotype machine with the help of a capacitor acting as a voltage regulator circuit.

Figure 7 – 400V, 150UF Capacitor

Function of Rectifier Diode. The AC voltage is converted to DC voltage in the power supply unit with a rectifier diode, which only enables the unidirectional flow of current. For most power supplies and electrical circuits needing DC voltage, rectifier diodes are essential.

Figure 8 – Rectifier Diode

Choke 5W. A Choke is a passive electronic component that saves energy in a magnetic field when a current flows. For the genotype machine power supply, a 5W choke can act in several ways, mainly when used with many other electronic parts to function as a voltage regulator, filter and store energy.

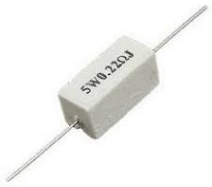

Figure 9 – Choke

The function of the AC Meter. The measurement of the AC voltage level in a circuit is done using an instrument known as an AC meter. The following are the functions of an AC meter in a genotype machine:

1. Measurement of Voltage: the AC voltage supplied to the haemoglobin electrophoresis machine is measured using an AC meter. For the machine to operate safely and efficiently, the AC meter helps to ensure that the voltage is within the acceptable range.

2. Monitoring Load: The AC voltage level under several load situations can be monitored by using an AC meter; this will help to ascertain if the instrument has a high current or if there is an issue with the circuit that needs to be solved.

3. Troubleshooting: An essential tool for checking and examining for electrical problems in the genotype machine is an AC meter. Through the voltage measurement at several points in the circuit, technicians can quickly identify areas where the voltage may be dropping or unnecessarily increasing, which indicates a potential issue with a particular component or connection.

4. Quality Control: An AC meter can also be used as part of a quality control measure to ascertain that the genotype machine is functioning as expected before being put to use.

Figure 10 – The AC Meter Voltage Display

The function of the switch. The ON/OFF control of the power supply unit of a haemoglobin electrophoresis machine is done using an electronic component called a switch. For conservation of power or safety reasons, a switch may be used to put on/off the power supply unit so the user can oversee the current flow to the genotype machine.

Figure 11 – A Switch

The Function of the Fuse. The fuse helps to protect against unexpectedly high current conditions in the power supply of a genotype machine; it also helps to ensure the safe and reliable operation of the machine.

Figure 12 – A Fuse

The function of the cable. Electrical power transmission within the genotype machine power supply from the power source to the electronic components is carried out using cables.

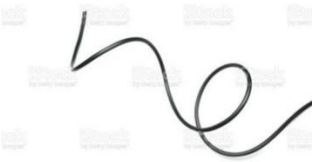

Figure 13 – Cable

Assembling of the Designed Genotype Machine. The power supply unit containing several components is connected to the electrophoretic chamber assembled to form the complete genotype machine.

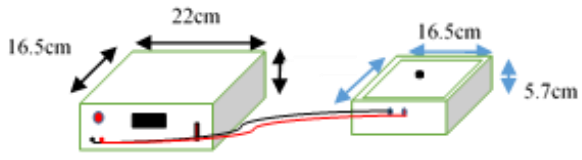

Figure 14 – The Block Diagram for the Genotype Machine

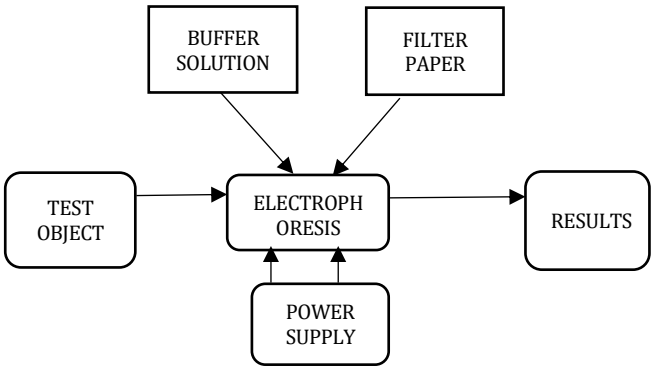

Figure 15 – Block Diagram of Electrophoresis Machine

Buffer Solution. The required medium for separating and analysing biomolecules in a cellulose acetate electrophoresis is provided with a buffer solution. The buffer solution protects the biomolecules from damage, enhances the visualisation of the genotype result, and helps maintain a stable pH with ionic strength. The buffer solution typically used in cellulose acetate haemoglobin electrophoresis is a Tris-borate-EDTA (TBE) buffer.

Filter Paper. Filter paper plays a crucial role in cellulose acetate haemoglobin electrophoresis by providing support for the cellulose acetate film, separating the haemoglobin variants, and facilitating the transfer of separated haemoglobin bands onto a detection membrane for visualisation and analysis.

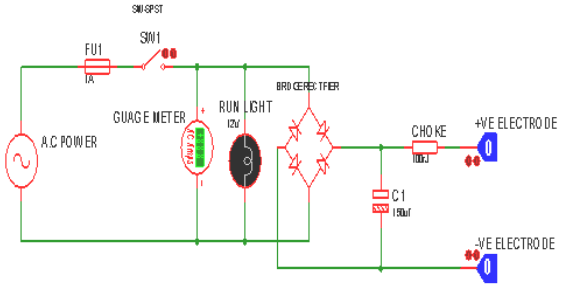

Figure 16 – The Circuit Diagram of the Constructed Power Supply Unit to the Electrophoretic Chamber

Table 1 – Average Cost of Component and Materials Used

No	Materials /Components	Cost (₦)
1	Capacitor 400V, 150UF	300
2	Choke 5W	400
3	Diode Rectifier	500
4	Power Switch	400
5	Running Light	600
6	White Plastic	2000

No	Materials /Components	Cost (₦)
7	Transparent Plastic	1000
8	Iron Sheet	3000
9	Cables	1000
10	Black Tape	500
11	AC Meter	2400
12	AV Cable	400
13	Workmanship	20,000
14	Gum, Blade and Others	5000
	Average Total Cost	₦29,500

RESULTS AND DISCUSSION

The test results on cellulose acetate paper are shown in Figures 17 to 25.

Test Procedure

1) Prepare a hemolysate of the patient samples as follows: *Using whole blood***:** Add 1 part whole blood to 3 parts Hemolysate reagent (distilled water is used). Mix well and allow to stand for 5 minutes.

2) Make a smear of the patient's samples with the control sample on the cellulose acetate paper to align the same line.

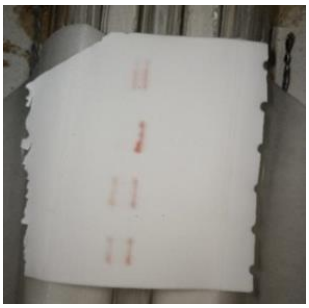

Figure 17 – AS, AA, AC, AC

Figure 18 – AC, AC, CC, SC

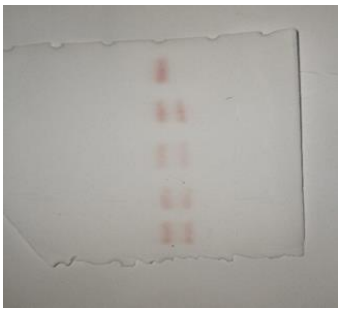

Figure 19 – SS, AS, AS, AS AS

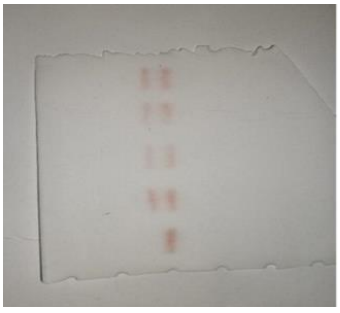

Figure 20 – AS, AS, AS, AS, AA

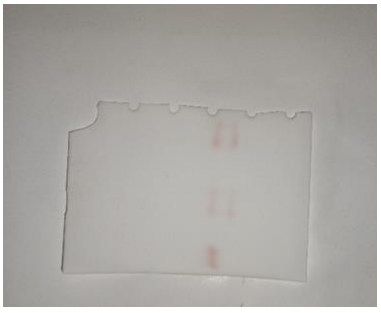

Figure 21 – AS, AS, SS

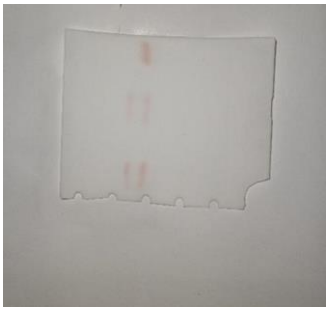

Figure 22 – AA, AS, AS

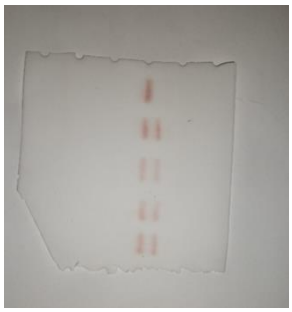

Figure 23 – SS, AS, AS, AS and AS

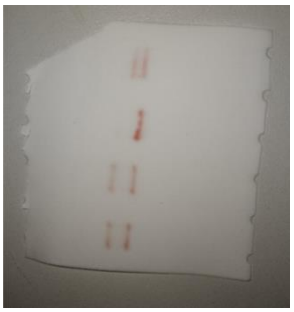

Figure 24 – AS, AA, AC and AC

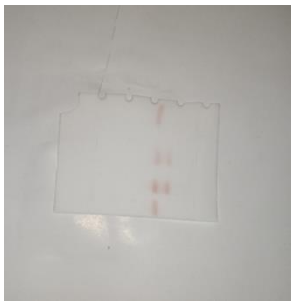

Figure 25 – SS, AS, AS and SS

3) The smeared cellulose acetate paper is placed on the two bridges inside the electrophoretic tank containing an alkaline buffer solution. (each bridge is mounted with filter paper)

4) The electrophoretic tank is covered.

5) The power supply unit is powered to supply voltage units ranging from 350v to 400v at 10 to 15 minutes.

6) The result from the cellulose acetate paper is produced, as seen in the pictures above, after 15 to 20 minutes of expiration.

7) The plates of haemoglobin can be examined for the presence of bands of haemoglobin that are not normal. Often, a patient sample of a known genotype is used as a control; this helps to guide the identification of the band.

NB: One or two control samples are used on each cellulose acetate paper. Known samples of AS AC are often used as controls.

Testing and Validation. Testing and validation of the low-cost electrophoresis machine using cellulose acetate paper is done to ensure the precision and efficiency of the machine in diagnosing genotype disorders.

The team took the following steps for testing and validation:

1. Analytical Validation: This involves testing the accuracy, precision, specificity, and sensitivity of the machine. Researchers used a well-characterised set of samples with known genotypes for this purpose.

2. Clinical Validation: This involves testing the machine with actual patient samples to confirm its ability to diagnose genotype disorders accurately.

3. Comparison with existing Machines: the result obtained was compared in the laboratory with those done using existing machines.

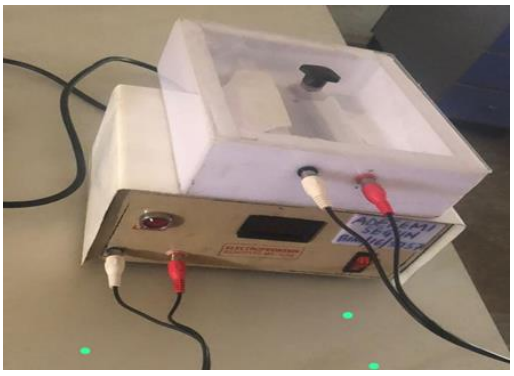

Figure 26 – The Constructed Genotype Machine

The Principle of Operation of the Genotype (Hemoglobin Electrophoresis) Machine. For the separation and identification of haemoglobin disorders, electrophoresis is majorly considered the best technique for diagnosis. The systems used for the process of haemoglobin electrophoresis are divided into two, which are the use of alkaline buffers use cellulose acetate as the primary support medium as this helps to produce smooth separation of Hb A, F, S and C together with several other mutants with short preparation time.

The presence of HbA, HbS, HbC and HbF, together with many other haemoglobin variants, is determined through a simple technique known as electrophoresis, which requires only a tiny amount of hemolysate.

Minutes of hemolysate samples are prepared from the blood collected from the patient and carefully applied on the cellulose acetate strip. Buffer solution at an alkaline pH of 8.2-8.6 inside the electrophoresis is used for the haemoglobin separation. The final result is read by comparing the band of the known samples, the control, with the unknown patient samples.

The design and construction of a low-cost haemoglobin electrophoresis machine using cellulose acetate paper has the potential to provide an affordable and accessible option for the examination of hemoglobinopathies in resource-deprived settings. The prevalence of hemoglobinopathies across the world, which are a significant cause of morbidity and mortality, makes this project very important.

Diagnosing many haemoglobin diseases, such as sickle cell anaemia and thalassemia, is popularly carried out using the haemoglobin electrophoresis technique. This technique deploys the principle of separating the types of haemoglobin based on their charge and size using an electric field and a support medium. The support medium commonly used is cellulose acetate paper for haemoglobin electrophoresis because the haemoglobin variants are well separated and have good resolution, as seen in chapter four of this project.

Using locally sourced materials to construct a low-cost genotype machine is a valuable move towards enhancing easy access to this diagnostic methodology in a limited resource setting. The construction and design accommodate a power supply unit, buffer solution, cellulose acetate paper, filter paper, and other necessary materials. The design of the machine was simple, portable, and user-friendly, making it available for use in primary healthcare settings.

The machine was put to the test after construction, and the results received using samples of known haemoglobin variants genotypes showed that the machine was effective and precise for diagnosing hemoglobinopathies. The construction of this design (low-cost haemoglobin elec-

trophoresis machine) yielded considerable success and has the potential to uniquely impact the diagnosis and control of genotype disorder in resource-limited settings.

To sum up, there is an invaluable step towards increasing easy access to this essential diagnostic tool in low-resource settings following the design and construction of a low-cost haemoglobin electrophoresis machine; this will lead to improvement in the early diagnosis and control of haemoglobin disorder associated with genetic mutation, which will also enhance health therapy for affected people.

Table 2 – Results and Interpretations

No	Figures	Results	Full meaning
1	Figure 17	Hb AS	Haemoglobin AS
		Hb AA	Normal Adult Haemoglobin
	Control	Hb AC	Haemoglobin AC
		Hb AC	Haemoglobin AC
2	Figure 18	Hb AC	Haemoglobin AC
	Control	Hb AC	Haemoglobin AC
		Hb CC	Haemoglobin CC
		Hb SC	Haemoglobin SC
3	Figure 19	Hb SS	Haemoglobin SS
		Hb AS	Haemoglobin AS
	Control	HbAS	Haemoglobin AS
		HbAS	Haemoglobin AS
		Hb AS	Haemoglobin AS
4	Figure 20	Hb AS	Haemoglobin AS
		Hb AS	Haemoglobin AS
	Control	Hb AS	Haemoglobin AS
		Hb AS	Haemoglobin AS
		Hb AA	Hemoglobin AA
5	Figure 21	Hb AS	Haemoglobin AS
	Control	Hb AS	Haemoglobin AS
		Hb SS	Haemoglobin SS
6	Figure 22	Hb AA	Haemoglobin AA
	Control	Hb AS	Haemoglobin AS
		Hb AS	Haemoglobin AS
7	Figure 23	Hb SS	Haemoglobin SS
		Hb AS	Haemoglobin AS
	Control	Hb AS	Haemoglobin AS
		Hb AS	Haemoglobin AS
		Hb AS	Haemoglobin AS
8	Figure 24	Hb AS	Haemoglobin AS
		Hb AA	Haemoglobin AS
	Control	Hb AC	Haemoglobin AC
		Hb AC	Haemoglobin AC
9	Figure 25	Hb SS	Haemoglobin SS
		Hb AS	Haemoglobin AS
	Control	Hb AS	Haemoglobin AS
		Hb SS	Haemoglobin SS
		Hb AS	Haemoglobin AS

Comparative Cost of the Genotype Machine. The average cost of production of the constructed genotype machine is ₦29,500 compared with the cost of selling the genotype machine in chapter two, which is sold at 380,000 NGN.

The constructed genotype Machine can be placed on commercial sales at ₦40,000 when mass production is done. Considering the constructed genotype machine's cost-effectiveness, accuracy, and reliability, it can be deployed in hospital settings.

Table 3 – Explanation of Each Genotype Result

No	Genotype result	Explanation
1	Haemoglobin AA	Hb AA refers to the normal haemoglobin genotype in humans. In this case, an individual has two regular copies of the haemoglobin gene, one inherited from each parent.
2	Haemoglobin AS	Hb AS refers to the sickle cell trait haemoglobin genotype in humans. In this case, an individual has one normal haemoglobin gene (Hb A) and one abnormal haemoglobin gene that causes the production of sickle haemoglobin (Hb S).
3	Hemoglobin CC	A form of haemoglobin variant produced due to a genetic mutation leading to haemoglobin disorder is HbCC. People with this haemoglobin abnormality (HbCC) have both haemoglobin genes mutated. Patients with this may experience mild to severe hemolytic anaemia.
4	Haemoglobin SS	HbSS is a form of haemoglobin disorder that emanates from a genetic mutation affecting the beta-globin subunit. This haemoglobin is characterised as sickle cell disease, a genetic disorder that assumes sickle cell shape, breaking down and leading to many complications. Those with HbSS inherit a mutated gene from both parents.
5	Haemoglobin SC	Abnormal haemoglobin that comes from the mutation of a gene affecting both the beta-globin and the alpha-globin

No	Genotype result	Explanation
		of the haemoglobin subunits is haemoglobin SC. A copy of the haemoglobin S is inherited from either parent, and a copy of haemoglobin C is inherited from the second parent.
6	Haemoglobin AC	This type of genotype is also called the Hb AC trait of haemoglobin AC disease. People with this genotype may have symptoms close to those with Hb AS (sickle cell trait). The abnormal haemoglobin is inherited from one of the parents. Symptoms are often less severe.
7	Controls AS, AC	These are the control samples, which help to enhance and guide the reliability, validity, and interpretation of the test results.

CONCLUSIONS

This project is an achievable and practicable approach for determining and identifying haemoglobin genotype disorders. This approach is a cost-effective alternative and provides precision in identifying haemoglobin variants. For resource-limited settings where easy access to costly and advanced laboratory equipment is limited, the design and construction of this machine are simple, user-friendly, and ultimately very affordable to low-income resource centres. The success of this project showcases the possibilities and potential for innovation as well as cost-effective answers to immediate healthcare problems. In developing countries where haemoglobin genotype disorders are prevalent, this project and development can help to significantly improve public health, especially in regions with limited resources. The successful construction of this project will heighten the improvement of the diagnosis and control of haemoglobin genotype abnormalities in these regions and, in turn, improve patient quality of life.

To sum up, the low-cost haemoglobin electrophoresis (genotype) machine designed and constructed using cellulose acetate paper can significantly change genotype testing in low-resource settings, giving patients access to early, timely and accurate diagnoses. Moreover, this project is a basis for upcoming research and development to enhance easy access to healthcare and output for the masses.

Based on this project, make the following recommendations:

1) To ascertain and ensure the machine's precision and reliability, the team should conduct consistent and further testing and validation.

2) Engineers can incorporate additional features like automated interpretation and analysis of results to improve machine outcomes.

3) Manufacturers can reproduce the machine on a bigger scale to meet the increasing demand for the haemoglobin genotype test in healthcare settings.

4) Researchers can lower the cost of production through subsequent research and innovations without affecting or compromising the machine's quality.

5) Applicable stakeholders in medical research and related fields can support this by ensuring increasing usage through innovative improvements on the machine.

With these recommendations above, the low-cost haemoglobin electrophoresis machine using cellulose acetate paper can give room for an easy accessibility and affordability methodology of genotype testing, especially in resource-limited settings where conventional ways of testing may not be available.

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