DISTRIBUTION OF ABO AND RH (D) BLOOD GROUPS AMONG STUDENTS OF SODO OROMO, WALANE AND KISTANE IN HARBU CHULULE PREPARATORY AND SECONDARY SCHOOLS AT SADEN SODO DISTRICT, SOUTH WEST SHEWA, OROMIA, ETHIOPIA

MSc THESIS

FEYISA GARI WAKJIRA

OCTOBER 2016
HARAMAYA UNIVERSITY, HARAMAYA

Distribution of ABO and Rh (D) Blood Groups among Students of Sodo Oromo, Walane and Kistane in Harbu Chulule Preparatory and Secondary Schools at Saden Sodo District, South West Shewa, Oromia, Ethiopia

A Thesis Submitted to the Department of Biology, Postgraduate Program Directorate
HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of MASTER OF SCIENCE IN BIOLOGY

Feyisa Gari Wakjira

October 2016
Haramaya University, Haramaya

HARAMAYA UNIVERSITY
POSTGRADUTE PROGRAM DIRECTORATE

We hereby certify that we have read and evaluated this thesis entitled ***Distribution of ABO and Rh (D) Blood Groups among Students of Sodo Oromo, Walane and Kistane in Harbu Chulule Preparatory and Secondary Schools at Saden Sodo District, South West Shewa, Oromia, Ethiopia,*** prepared under my guidance by Feyisa Gari. We recommend that it be submitted as fulfilling the thesis requirement

Tamiru Oljira (PhD)	_____	_____
Major advisor	Signature	Date
Yohannes Petros (PhD)	_____	_____
Co-Advisor	Signature	Date

As a member of the Board of Examiners of the MSc Thesis Open Defense Examination, we certify that we have read and evaluated the Thesis prepared by Feyisa Gari and examined the candidate. We recommend that the thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in Biology

Ameha Kebede (PhD)	_____	_____
Chairman	Signature	Date
Meseret Chimdessa (PhD)	_____	_____
Internal examiner	Signature	Date
Kifle Dagne (PhD)	_____	_____
External examiner	Signature	Date

Final approval and acceptance of the Thesis is contingent upon the submission of its final copy to the Council of Graduate Studies (CGS) through the candidate's department or School graduate committee (DGC or SGC).

DEDICATION

I dedicate this Thesis manuscript to my mother **Warki Nagawo Bedane** who laid the foundation to all my life since my early stage of childhood and devoted much in nursing me with special affection and love throughout my life. Also my wife **Tolashi Adare** for her love and moral support in my entire career through my education for the success of my life.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis, and completion of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation. This thesis is submitted in partial fulfillment of the requirements for an MSc degree in Biology at Haramaya University. The Thesis is deposited at the Haramaya University Library and is made available to borrowers under the rules of the Library. I solemnly declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Brief quotations from this Thesis may be made without special permission provided that accurate and complete acknowledgement of the sources is made. Requests for permission for extended quotation from or reproduction of this Thesis in whole or in part may be granted by the Head of the School or the Department when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author of the Thesis.

Name: Feyisa Gari Wakjira

Signature: _____

Department: Biology

Date of submission: _____

BIOGRAPHICAL SKETCH

Feyisa Gari was born on August 21, 1988 in Bele Gesgar Woreda, Genale Negaha (Oda Kole) Kebele, East Arsi Zone, Oromia Regional State. He attended his Elementary school education at Hibrat Primary school (1-3) in 1998-1999 and Koshimo Junior School (4-8) in 2000-2004, Aymero Bele Secondary School (9-10) in 2005-2006 and Preparatory School at Didea Preparatory School, Arsi Robe, in 2007-2008. He joined Madawalabu University in 2009 and received his first degree (Bachelor of Science) in Biology in 2011. He also worked as a Biology teacher at Saden Sodo Woreda in Harbu Chulule Preparatory School for two years. He then joined Haramaya University in 2013 to pursue MSc in Biology.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my advisors, Tamiru Oljira (PhD) and Yohannes Petros (PhD) for their consistent valuable advice, comments and follow up right from start to the completion of my work.

I would like to thank the Harbu Chulule Preparatory and Secondary School students at Saden Sodo district for their kindness for participating in the blood test, director of Harbu Chulule Preparatory School, Mr. Birhanu Gebrasilassie for his moral support, Nugusie Dibaba and Baharu Lama for their help testing blood and generating quality data; my colleagues Fasika Yohannes, Dejene Teddessa, Tedela Tekela, Geremo Tedessa, Dula Qumbi and Deferu Heyi for their moral support.

I am very much indebted to my mother Warki Nagawo and my wife Toleshi Adare and my sisters Shurabe and Bose Gari for their moral support and encouragements in the course of my study.

ACRONYMS AND ABBREVIATIONS

HDN	Hemolytic Disease of the Newborn
HWP	Hardy-Weinberg Principles
Ig	Immunoglobulin
ISBT	International Society of Blood Transfusion
OLECSSD	Office of Land Management and Environmental Conservation of Saden Sodo District
RBCs	Red Blood Cells
Rh	Rhesus

TABLE OF CONTENTS

Table of contents (*Contuened*)

LIST OF TABLES

LIST OF FIGURES IN THE APPENDIX

Distribution of ABO and Rh (D) Blood Groups among Students of Sodo Oromo, Walane and Kistane in Harbu Chulule Preparatory and Secondary Schools at Saden Sodo District, South West Shewa, Oromia, Ethiopia

ABSTRACT

The ABO and Rh (D) blood groups are the hereditary characters and are useful in population genetic studies, in resolving medico-legal issues and more importantly in compatibility test in blood transfusion practice and they are the most important blood group system in human blood transfusion and forensic investigation. The Aim of the study was to investigate the distribution of ABO and Rh (D) blood groups among students of three ethnic groups in Harbu Chulule Preparatory and Secondary School at Saden Sodo District, South West Shewa, Oromia, Ethiopia. Harbu Chulule Preparatory and Secondary School had 819 students enrolled for the academic year 2015/2016. Among them 351 (42.9%) were females and 468 (57.1%) were males. The study was conducted on 384 students. These were purposively selected and the sample was divided into Sodo Oromo, Walane and Kistane and stratified along ethnic lines. Allele frequencies were calculated under the assumption of Hardy-Weinberg principles. The overall allelic frequencies of the population as they were calculated using the extension of the Hardy–Weinberg law were 0.59, 0.21 and 0.20 for I^O, I^A and I^B alleles, respectively. Also on the Rhesus status, the allelic frequencies were 0.81 and 0.19 for D and d alleles, respectively. Chi-square test was used to compare observed phenotypic frequency distribution of ABO and Rh (D) blood groups with that expected under the Hardy-Weinberg law. The Percentage distribution of the ABO blood group among the three ethnic groups were 30.47%, 27.34%, 7.81% and 34.38% for blood group A, B, AB and O, respectively. The most prevalent blood group was type O followed by A, B, and AB. Blood group O had the highest distribution while blood group AB had the least distribution. The percentage distribution of Rh (D) blood group among the three ethnic students were 96.35% and 3.65% Rh^+ and Rh^- in the population of Saden Sodo district respectively. The ABO and Rh (D) blood groups vary among students of *three ethnic groups*.

Key wards: Allelic frequency, Genotype, Phenotype, Red Blood Cells, Rhesus

1. INTRODUCTION

The ABO and Rh (D) blood groups are hereditary characters and are useful in population genetic studies, in resolving medico-legal issues and more importantly in compatibility test in blood transfusion practice and they are the most important blood group systems in human blood transfusion and forensic investigations (Alimba *et al*., 2010). The ABO blood types are also present in some other animals, for example rodent and apes, such as chimpanzees, bonobos, and gorilla (Maton, 2014).

The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who found three different blood types in 1900 (Garratty *et al*., 2000). He described A, B and O blood groups for which he was awarded the Nobel Prize in 1930. Alfred Von Decastello and Adriano Sturli discovered the fourth type AB in 1902 (Von *et al*., 1902).

There are many blood group systems on the basis of different blood group antigens, but only ABO and Rh (D) blood groups are the most important in clinical practice (Seely *et al.*, 1998). The ABO blood group consists of four main blood groups: A, B, AB and O which are determined on the basis of the presence or absence of two antigens: A and B antigens (Landsteiner, 1990).

These antigens may be proteins, carbohydrates, glycoproteins, and glycolipids depending on the blood group system and some of these antigens are also present on the surface of other types of cells which are under the control of three allelic genes, namely I^A, I^B, and I^O and that I^A allele produces the A antigen, the I^B produces antigen B, and I^O produces neither antigen. The I^A and I^B alleles are co-dominant but both of them are dominant the recessive allele I^O (Povey *et al.*, 1978).

Because of the presence of several antigens on the surfaces of RBCs it is not possible to mix the blood of all humans without initiating an immune reaction. Only the blood sample, which share the same antigenic identity do not initiate an immune response

and hence are termed as compatible. The utility of these antigens is not only for blood transfusion or organ transplantation, but have also been utilized in genetic research, anthropology and tracing of ancestral relation to human beings (Khurshid *et al*.,1992).

The human red blood cells have a series of glycoproteins and glycolipids on their surfaces of which constitute the blood group antigens and contains different types of polysaccharide antigens called agglutinogen (Ganon, 1995). The antigenic substances are capable of inducing specific immune response results in the production of antibodies (Novak, 1995). In addition, the presence of Rh (D) blood group system was recognized in 1939 and it was confirmed within few years (Landsteiner and Weiner, 1940).

The Rh^+ and Rh^- in Rh (D) blood group system are based on the presence or absence of inherited antigenic substances (D gene which is determined by two alleles: D and d) on the surface of the red blood cells. Identification of Rh (D) blood group system is important to prevent the erythroblastosis fetalis; which commonly arises when an Rh^- mother carries an Rh^+ fetus. The Rh factor assumes a special importance in maternal-fetal interactions. A mother who is Rh^- can bear an Rh^+ child if the father is Rh^+ (either homozygous or heterozygous). Since there are no natural anti-Rh antibodies, this generally poses no special risk for the first pregnancy (Enosolease and Bazuaye, 2008).

The need for blood group prevalence studies is of multipurpose; besides their importance in evolution, their relation to disease and environment is being increasingly sought in modern medicine (Green *et al.*, 1995).

The human blood groups have been studied extensively for their involuntary compatibility solutions. Red blood cells have a series of glycoproteins and glycolipids on their surfaces which constitute the blood group antigens (Srikumeri *et al.*, 1987).

In Ethiopia, several of ABO and Rh (D) blood group studies have been conducted in different Zones and Universities. However, no investigation was made in the

population of Saden Sodo District. Therefore, the aim of this study was to investigate the distribution of ABO and Rh (D) blood groups among students of Sodo Oromo, Walane and Kistane in Harbu Chulule Preparatory and Secondary School at Saden Sodo District, South West Shewa, Oromia, Ethiopia, with the following objectives.

Objectives of the study

General objective

The general objective of the study was to describe the distribution of ABO and Rh (D) blood groups among students of Sodo Oromo, Walane and Kistane in Harbu Chulule Preparatory and Secondary School at Saden Sodo District of South West Shewa Zone of Oromia Regional State in Ethiopia.

The specific objectives are:

- To determine ABO and Rh (D) blood group phenotypes among students of the three ethnic groups.
- To estimate the allelic frequencies of ABO and Rh (D) blood groups for each ethnic groups and for the whole population from the phenotypes assuming Hardy-Weinberg equilibrium.
- To estimate the genotype frequency for each group and for the whole population.
- To determine if the populations are in Hardy-Weinberg equilibrium or not.

2. LITERATURE REVIEW

2.1. History of ABO and Rh (D) Blood Group Systems

In 1901, an Austrian scientist Karl Landsteiner established the existence of the first known blood group system (Garratty *et al*., 2000). Landsteiner named the first two blood groups, antigen A and antigen B, using the first two letters of alphabet while RBCs not reacting with anti-A and anti-B were called type C. Classification of the blood group was based on his observation of the agglutination reaction between an antigen on erythrocytes and antibodies presents in the serum of individuals directed against this antigen. Where no agglutination had occurred, either antigen or antibody was missing from the mixture. In 1902 Von Decastello and Sturil (Greenwalt, 1997) described RBCs reacting with both anti-A and anti-B, but did not give these type a name, but continued calling RBCs that did not react with anti-A and anti-B type C (Garratty *et al*., 2000).

In 1911, von Dungern and Hirszfeld (Griffithis *et al.,* 2008) were the first to use the term O to describe RBCs not reacting with anti-A and anti-B and the term AB for RBCs reacting with both anti-A and anti-B (Millison, 1994).

The Rh (D) blood group is named after the Rhesus monkey, *Macaca mulatta* (Zimmerman) in which the Rh antigens were discovered in 1940. This group is determined by a gene called D which has two alleles: D, d. Whatever other alleles a person may have, anyone with genotype DD or Dd has D antigens on his or her RBCs and is classified as Rh-positive (Rh^+). In Rh-negative (Rh^-) people, the D antigen is lacking. The Rh blood type is tested by using an anti-D reagent. In the ABO blood groups, anti-D antibodies are not normally present in the blood. They form only in Rh^+ individuals who are exposed to Rh^- blood. If an Rh^- person receives an Rh^+ transfusion, the recipient produces anti-D. A related condition sometimes occurs when an Rh^- woman carries an Rh^+ fetus. The first pregnancy is likely to be uneventful because the placenta normally prevents maternal and fetal blood from mixing. However, at the time of birth, or if a miscarriage occurs, placental tearing exposes the mother to Rh^+ fetal blood. She then begins to produce anti-D antibodies. If she becomes pregnant again

with an Rh^+ fetus, her anti-D antibodies may pass through the placenta and agglutinate the fetal erythrocytes. Agglutinated RBCs hemolyze, and the baby is born with a severe anemia called hemolytic disease of the newborn (HDN), or erythroblastosis fetalis (Saladin, 2003).

2.2. Blood Group Systems

2.2.1. ABO blood grouping

The ABO system is the most important blood group system in human blood transfusion. The associated of anti- A and anti- B antibodies are usually immunoglobulin M, abbreviated IgM, antibodies. ABO IgM antibodies are produced in the first years of life by sensitization to environmental substances such as food, bacteria and viruses. The O in ABO is often called 0 (zero or null) in other languages (Chou *et al*., 2010).

The classification of blood groups into A, B, AB, and O in ABO blood group system, is based on the presence or absence of inherited antigenic substances on the surface of red blood cells. The ABO blood group distribution varies among the difference racial and ethnic groups all over the world. For example, blood group B has its highest frequency in North India and neighboring central Asia, and its incidence diminishes both towards the West and the East, falling to single percentages in Switzerland. It is believed to have been entirely absent from native America and Australia aboriginal population prior to the arrival of Europeans in this areas. Blood group A is associated with high frequencies in Europe, especially in Scandinavia and central Europe, although their highest frequencies occur in some Austrian aborigine population and the black foot India of Montana (ISBT, 2006). The antigens may be proteins, carbohydrates, glycoproteins and glycolipids depending on the blood group system (Hasna *et al.*, 2010).

Almost always, an individual has the same blood group for life, but very rarely an individual's blood type change through addition or suppression of an antigen in infection, malignancy, or autoimmune disease. Another more common blood type

change is bone marrow transplant. Bone marrow transplants are performed in many leukemias and lymphomas, among other disease (Masushita *et al.*, 1983). If a person receivers bone marrow from someone who is different ABO type (e.g., a type A patient receives a type O bone marrow). The patient's blood type will eventually convert to the donor's type (Masushita *et al.*, 1983).

Certain blood types may affect susceptibility to infection, an example being the resistance to specific malaria species seen in individuals lacking the Duffy antigen. The Duffy antigen, presumably as the result of natural selection, is less common in ethnic groups from areas with high incidence of malaria (Chown *et al.*, 1957).

2.2.2. Rh (D) blood grouping

The Rh (D) blood group is one of the complex blood groups known in humans. It is one of thirty current human blood group systems. Clinically it is the most important blood group system after ABO at present. The Rh (D) blood group systems consist of 50 defined blood group antigens, among which the five antigens D, C, c E, and e are the most important (Daniels, 2002).The commonly used terms Rh factor, Rh positive and Rh negative refer to the D antigen only. Beside its role in blood transfusion, the Rh (D) blood group system, specially the D antigen, is used to determine the risk of hemolytic diseases of the newborn (HDN) (Reid and Lomas, 2004).

An individual either has, or does not have the Rhesus factor on the surface of their red blood cells. This term is strictly refers only to the most immunogenic D antigen of the Rh^+ blood group system, or the Rh- blood group system. The status is usually indicated by positive (Rh^+ does have the D antigen) or Rh negative (Rh^- does not have the D antigen) suffix to the ABO blood type. However, other antigens of this blood group system are also clinically relevant. Unlike the anti-A and anti-B antibodies, anti-D antibodies are only seen if a patient lacking D antigens is exposed to D^+ cells. The exposure of D^+ cells usually occurs through pregnancy or transfusion. In contrast to the ABO blood group, immunization against Rh can generally only occur through blood transfusion or placental exposure during pregnancy in women (Daniels, 2005).

2.3. Blood Typing

2.3.1. ABO blood typing

The ABO system is regarded as the most important blood group in transfusion medicine because of severe hemolytic transfusion reactions and, to a lesser degree hemolytic disease of the newborn. The presence of these antigens and antibodies can be readily detected by the agglutination reaction; mixing type A plasma (which contains anti-B antibodies) with type B red blood cells, for instance, results in agglutination of the red cells which can be easily observed. The blood type of any given individual can be determined in this manner, carrying out the agglutination reaction with a set of standard antibody-containing sera. Blood typing involves identifying antigen present on red blood cell (RBC) membranes. Many different antibodies exist on human RBC and Rh groups. A person normally produces antigens against those antibodies not present on his/her RBCs but does not produce antibodies against those that are not present. Thus a person with antigen A has anti B antibodies, etc. Blood type is determined by multiple alleles I^AI^B and I^OI^A and I^BI^O which are co dominant and all are dominant over O. Genotype I^AI^A and I^AI^B result in blood type A. Genotypes I^BI^B result in blood type B. When both I^A and I^B are present, the blood type is AB. When both alleles are O, the blood type is O (Avent, 2009).

In addition to the current practice of serological testing of blood types, the progress in molecular diagnostic allows the increasing use of blood group genotyping. In contrast to serological tests reporting a direct blood group type phenotype, genotyping allows the prediction of phenotype based on knowledge of molecular basis of the currently known antigen. This allows a more detailed determination of the blood type and therefore a better match for transfusion, which can be crucial in particular for the patients with needs for much transfusion to allow for the immunization of all (Anstee, 2009).

Table 1. ABO blood system (Avent, 2009)

Blood type	Antigens on erythrocytes	Antibodies in plasma	Can receive blood from groups	Can give blood to groups
A	A	B	O, A	A, AB
B	B	A	O, B	B, AB
AB	A and B	None	O, A, B, AB	AB
O	None	A and B	O	O, A, B, AB

Blood typing is performed with anti-sera blood serum that contains specific antibodies. For ABO blood typing antibodies against A and B antigen (these antibodies are also called anti-A and anti- B antibodies) are used. If clumping or clotting occurs in the test blood upon exposure to the A antibodies (anti-A serum) the blood contains the A antigen (Table 2). If clumping or clotting occurs in the test blood upon exposure to the B antigens (anti-B serum) the blood contains the B antigen. If clotting occurs with both A and B antibodies (anti- A and anti- B sera) the type is AB and if no clumping occurs with either serum type, type is O (Avent, 2009).

Table 2. Agglutination reaction of ABO blood typing serum (Avent, 2009**)**

Reaction		Blood type
A antibodies (anti-A serum)	B antibodies (anti-B serum)	
Clumping	No clumping	Type A
No clumping	Clumping	Type B
Clumping	Clumping	Type AB
No clumping	No clumping	Type O

2.3.2. The Rh (D) blood typing

While many blood group systems are known other than the ABO system, the Rh system is of special importance. This was originally defined by a rabbit antibody directed against the red blood cells of Rhesus monkeys, an antibody which turned out to be capable of distinguishing between the red blood cells of different human individuals. In simple terms, this system is defined by the presence or absence of a single red blood cell antigen, representing the two blood types Rh^+ and Rh^- (Saladin, 2003).

These are determined by two alleles at a single locus, which segregate independently of the ABO blood group locus. Thus an Rh^+ individual may be homozygous ($^+/^+$) or heterozygous ($^+/^-$), while an Rh^- individual must be homozygous ($^-/^-$). However, unlike the A and B antigens, the Rh antigens are present only on red blood cells. Therefore, while they are important for blood transfusion, they do not normally play a role in organ transplantation, and Rh typing of organ donors and recipients (Saladin, 2003).

2.4. Clinical Significance of Blood Groups

2.4.1. Blood transfusion

Transfusion is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of blood bank to provide transfusion services for blood and other blood products. Across the world, blood product must be prescribed by a medical doctor in a similar ways as a medicine. Much of the routine work of the blood bank involves testing blood from both doctors and recipients to ensure that every individual recipient is given blood that is compatible and is as safe as possible. If a unit of incompatible blood is transfused between a doctor and recipient sever acute hemolytic reaction with hemolytic (RBC destruction), renal failure and shock is likely to occur and death is a possibility. Antibodies can be highly active and can attach RBCs and bind components of the complement system to cause massive hemolysis of the transfused blood (Nickel *et al.*, 1999).

Patients should ideally receive their own blood or type specific blood products to minimize the chance of transfusion reaction. Risks can be further reduced by a cross-matching blood, but this may be skipped when blood is required for an emergency. Cross matching involves mixing a sample of the recipient's serum with a sample of the red blood cells and shacking if the mixture agglutinates, or form clumps. If agglutination is not obvious by direct vision, blood bank technicians usually check for agglutination within a microscope. If agglutination occurs, that particular doctor's blood cannot be transfused to that particular recipient (Bruce, 2002).

2.4.2. Hemolytic disease of the newborn (HDN)

A pregnant woman can make IgM blood group antibodies if her blood antigen that she does not have. This can happen if \some of the fetus blood cells pass into the mothers blood circulation (e.g. a small fetus maternal hemorrhage at time of childbirth or obstetric intervention), or some times after therapeutic blood transfusion. This can cause Rh disease or other form of hemolytic disease of the newborn in the current pregnancy and /or subsequent pregnancies (Letsky *et al.*, 2000).

If a pregnant woman is known to have anti-D antibodies, the Rh blood type of a fetus can be tested by analysis of fetal DNA in maternal plasma to assess the risk to the fetus of Rh disease. Antibodies associated with some blood groups can cause severe HDN, other can only cause mild HDN and other is not known to cause HDN (Letsky *et al.*, 2000).

Anti-D cause the most severe form of HDN and it used to be a major cause of fetal death. Since the introduction of ant-D immunoglobulin along with careful monitoring of at-risk pregnancies, the prevalence of HDN because of Rh (D) incompatibility has decrease dramatically. However, all cases cannot be prevented and Rh (D) all immunization remains a major cause's disease (Urbaiac *et al.*, 2000).

2.4.3. Red blood cells compatibility

Blood group A individuals have the A antigen on the surface of their red blood cells and blood serum containing IgM antibodies against the B antigen. Therefore, a group A individual can receive blood only from individuals of A or O (with being preferable). And can donate blood to individual with type A or AB (Simpkins and Williams, 1997).

Blood groups B individuals have the B antigen on the surface of their red blood cells and blood serum containing IgM antibodies against the antibodies A antigen. Therefore, a group B individual can receive blood only from individuals of groups B or

O (with B being preferable). And can donate blood to individuals with type B or AB (Bruce, 2002).

Blood group AB individual have both A and B antigens on the surface of their red blood cells and their blood serum does not contain any antibodies against either A or B antigen. Therefore, an individual with type AB blood group can receive blood from any group (with AB being preferable). But can donate blood only to another AB individual (Greenwalt, 1997).

Blood group O individual (whose red cells bear neither antigen) is classically referred to as a universal donor, since this blood can fairly safely be given to a recipient with any ABO type. But their blood serum contains IgM anti-A antibodies and anti-B antibodies against the A and B blood group antigens. Therefore, a group O individual can receive blood only from a group O individual. If anyone needs a blood transfusion in a dire emergency and if the time taken to process the recipient's blood would cause a determine delay O negative blood can be issued (Griffithis *et al.*, 2008).

Table 3. Summary of Red Blood Cell Compatibility

		Donors							
		O^+	A^+	B^+	AB^+	O^-	A^-	B^-	AB^-
Recipients	O^+	✔				✔			
	A^+	✔	✔			✔	✔		
	B^+	✔		✔		✔		✔	
	AB^+	✔	✔	✔	✔	✔	✔	✔	✔
	O^-					✔			
	A^-					✔	✔		
	B^-					✔		✔	
	AB^-					✔	✔	✔	✔

2.5. Population Genetics of ABO and Rh (D) Blood Groups

2.5.1. Distribution of ABO blood groups

The ABO blood group types vary in distribution worldwide and are not found in equal numbers even among different ethnic groups (Table.3). The distribution of the blood groups A, B, O and AB varies across the world according to the population. The A blood allele is somewhat more common around the world than B. About 21% of all people share the A allele. The highest frequencies of A are found in small, unrelated populations, especially the Blackfoot Indians of Montana (30-35%), the Australian Aborigines (many groups are 40-53%), and the Lapps, or Saami people, of Northern Scandinavia (50-90%). The A allele apparently was absent among Central and South American Indians (Ember *et al*., 2010).

We have learned a good deal about how common each of the ABO blood type is around the world. It is quite clear that the distribution patterns are complex. Both cline and discontinuous distributions exist, suggesting a complicated evolutionary history for humanity. This can be seen with the global frequency patterns of the type B blood allele. Note that it is highest in Central Asia and lowest among the indigenous peoples of the Americas and Australia. The O blood type is very common around the world. About 63% of humans share it. Type O is particularly high in frequency among the indigenous populations of Central and South America, where it approaches 100%. It also is relatively high among Australian Aborigines and in Western Europe (especially in populations with Celtic ancestors). The lowest frequency of O is found in Eastern Europe and Central Asia, where B is common. In Caucasians in the United State, the distribution is that type O, (47%), type A, (41%), type B, (9%), type AB, (3%). Also, among Western Europeans, type O, (46%), type A, (42%), type B, (9%), and type AB, (3%) (Adeyemo *et al*., 2006). However, there are relatively high frequency pockets in Africa as well. Overall in the world, B is the rarest ABO blood allele. Only 16% of humanity have it (Ember *et al*., 2010).

For instance, in African- American ABO blood group, the distribution of type O, (46%), type A, (27%), type B, (20%), and type AB, (7%). Also Bakare *et al*. (2005)

report showed that (22.9%) were blood group A, (21.3%) were blood group B and (5.9%) was blood group AB and (50.0%) were blood group O. Similarly, O blood group has the highest overall percentage frequency (67.26%) among a Nigerian population while AB blood group has the least overall percentage frequency (3.10%) (Alimba *et al*., 2010). In Nigeria, among 7653 individuals in Ogbomoso, Oyo State, (50%) had type O, type A, (22.9%), type B, (21.3%) and type AB, (5.9%) (Bakare *et al*., 2006).

Table 4. Distribution of ABO blood frequencies in different populations across the world

Country	A (%)	B (%)	AB (%)	O (%)	References
Cameroon	25	22	4	49	Hamed *et al*., (2012)
Egypt	30	24	8	33	Tagny *et al*., (2012)
Ethiopia	27	25	5	43	Bloodbook.com. Retrieved, (2010)
Kenya	16	13	2	69	Lyko *et al*., (1992)
Madagascar	23	30	6	42	Loua *et al*., (2007)
Nigeria (Ogbomo)	23	21	6	50	Bakare *et al*., (2006)
Sudan	18	12	3	67	Khalil *et al*., (1989)
South Africa	40	11	4	45	Garratty *et al*., (2004).
Britain	42	9	3	47	Khattak *et al*., (2008)
Hungary	28	12	4	60	Tuasik, (1995)
Turkey	12	12	8	74	Akbas *et al*., (2003)
India	19	33	10	39	Khattak *et al*., (2008)
Pakistan	17	22	4	56	Anees *et al*., (2005)

2.5.2. Distribution of Rh (D) blood groups

The majority of the people in the world have the Rh^+ blood type. However, it is more common in some regions. Native Americans and Australian Aborigines were very likely (99-100%) Rh^+ before they began interbreeding with people from other parts of the world. This does not imply that Native Americans and Australian Aborigines are historically closely related to each other. East Asians are (93-99%) Rh^+. Europeans have the lowest frequency of this blood type for any continent; they are 83-85% Rh^+.

The lowest known frequency is found among the Basques of the Pyrenees Mountains between France and Spain; they are only (65%) Rh^+. Most Sub-Saharan African populations are around (97-99%) Rh^+. Moreover, Rh^+ is documented as (95%) in African-Americans, (100%) in Africans whereas Rh^- is (5.5%) in South India, (5%) in Nairobi, (7.3%) in Lahore and (4.8%) in Nigeria (Greer *et al*., 2010).

Table 5. Distribution of Rh (D) blood studied in different populations across the world

Country	Rh^+ (%)	Rh^- (%)	References
Britain	83	17	Khattak *et al*., (2008)
Cameroon	96	4	Hamed *et al*., (2012)
Egypt	92	8	Tagny *et al*., (2012)
Ethiopia	93	7	Bloodbook.com. Retrieved, (2010)
India	94	6	Das *et al*., (2001)
Kenya	96	4	Lyko *et al*., (1992)
Madagascar	99	1	Loua *et al*., (2007)
Ogbomoso (Nigeria)	97	3	Bakare *et al*.,(2006)
Pakistan	91	9	Khurshid *et al.*, (1992)
USA	85	15	Khattak *et al*., (2008)
Saudi Arabia	93	7	Khattak *et al*., (2008)
Turkey	86	14	Akbas *et al*., (2003)

2.5.3. Significance of the distribution of ABO and Rh (D) blood groups

The success of human blood transfusions requires compatibility for the two major blood group antigen systems, namely ABO and Rh. Identification of ABO and Rh (D) blood group distribution among populations has various benefits in transfusion medicine, transplantation and disease risk assessment. The knowledge of distribution of ABO and Rh (D) blood groups at local and regional levels are helpful in the effective management of blood banks and safe blood transfusion services. Furthermore, the discovery of ABO and Rh (D) blood groups has contributed immensely to blood banking services and transfusion medicine in order to avoid morbidity and mortality in both adults and children (Fareed *et al*., 2014).

Also ABO distribution study is performed in order to prevent an adverse transfusion reaction that could be caused by ABO incompatibility between patient and a blood donor. For a blood donor and recipient to be ABO-compatible for a transfusion, the recipient must not have Anti-A or Anti-B antibodies that correspond to the A or B antigens on the surface of the donor's red blood cells (since the red blood cells are isolated from whole blood before transfusion, it is unimportant whether the donor blood has antibodies in its plasma). If the antibodies of the recipient's blood and the antigens on the donor's red blood cells do correspond, the donor blood is rejected (Fareed *et al*., 2014).

2.5.4. Extension of Hardy-Weinberg law to loci with more than two alleles

The Hardy-Weinberg model describes a mathematical relationship that allows the prediction of the frequency of offspring genotypes based on parental allele frequencies. It also predicts that allele frequencies will not change from one generation to the next, indicative of non-evolution (Klug & Cummings, 2002; Mayo, 2008).

For a population to be in Hardy-Weinberg equilibrium, the following assumptions are required to hold: random mating, no mutation, no migration, no stochastic effects or genetic drift due to small population size, and equal fertility for all genotype groups so that no selection is occurring (Muhammad *et al.,* 2010). Violation of any of these assumptions can result to evolutionary change in terms of allelic frequency distribution (Mayo, 2008). These conditions, however, seldom occur simultaneously, resulting to most populations not exhibiting Hardy-Weinberg equilibrium and are therefore evolving. When two allele are present at a locus, the Hardy- Weinberg law tells us that equilibrium in the frequencies of the genotypes is $p^2 + 2pq + q^2$, which is the square of allelic frequencies $(p + q)^2$. This is the simple binomial expansion and this principle of probability theory can be extended to any number of alleles that alleles are sampled two at a time into a diploid zygote (Daniel *et al.,* 2007). It is also expressed using Punnet square as follows for more than two allele.

Table 6. Summary showing Hardy-Weinberg frequencies for the ABO blood groups

By using this formula: $(p + q + r)^2$

Male gamete

Allele	I^A	I^B	I^O
Frequency	p	q	r

I^AI^A p^2	I^AI^Bpq	I^AI^Opr
I^AI^B pq	$I^BI^Bq^2$	I^BI^O qr
I^AI^O pr	I^BI^B qr	I^OI^O r^2

Female gamete

Allele	frequency
I^A	p
I^B	q
I^O	r

3. MATRIALS AND METHODS

3.1. Description of the Study Area

The study was carried out in Harbu Chulule Preparatory and Secondary School at Saden Sodo District, South West Shewa Zone, Oromia Regional State, Ethiopia. It is situated at a distance of 62 km away from Waliso (Zonal Capital) and 107 km away from Addis Ababa in South West direction. It lies at 8^0 16" to 8^0 36"N, and 38^0 8" to 38^0 28" East. The altitude of Saden Sodo district ranges from 2300 to 3543 meters above sea level. It is bordered by Bacho district (Oromia) in the north, by Malamba (Southern Nations Nationalities and Peoples) in the south by Waliso district (Oromia) in the west and by Qarsa Mallima district (Oromia) in the east. The temperature of Saden Sodo district is 10.94^0C-27.78^0C and the mean annual rain fall is 1286.6 mm. Saden Sodo district is established on 49,169 hectare and has 93,126 population size in 2015 (OLECSSD, 2011).

Sodo Oromo population size is 74,338, Walane is 9,175 and that of Kistane population 9,613 out of the total population district. Sodo Oromo have three races: Oditu, Tume and Liben; those are known as Saden Sodo. Walane and Kistane population located at the Gurage zone but their culture almost similar while their language is Afan Oromo in Sodo Oromo, Walanign for Walane, Kistanign for Kistane (OLECSSD, 2015).

3.2. Participants of the Study

Harbu Chulule Preparatory and Secondary School had 819 students enrolled for the academic year 2015/2016. Among them 351 (42.9%) were females and 468 (57.1%) were males. The study was conducted on 384 students comprising approximately 46% of the student population. From 384 students, 178, 102 and 104 were Sodo Oromo, Walane and Kistane, respectively, which show the number of Walane and Kistane ethnic groups less than that of Sodo Oromo. Individuals were purposively selected and the information about the ethnic group was provided by students themselves when filling their personal profile form, before conducting blood test. Therefore, ethnicity was self-declared and stratified along ethnic lines.

3.3. Blood Sample Collection Procedure

Blood sample was collected from each volunteer students which include both genders and blood was collected by a lab technician. The ABO and Rh (D) blood group tests were performed by using sterilized needles and packed lancet, to obtain a drop of blood from finger. Blood sample was taken from finger prick after wiping the finger with a piece of cotton saturated with 70% alcohol and piercing it with a sterile disposable lancet. Then, it was placed on each of three clean slides with few drops of one of the anti-sera, that is, antibody coated anti-A, anti-B, and anti-D [Manufactured by Tulip Diagnostic (P) Limited, Old Goa, India] were added to blood droplets and mixed using a glass rod. Blood group was determined on the bases of agglutination and recorded, as blood A^+, B^+, AB^+, O^+, or A^-, B^-, AB^-, O^-. The blood samples were collected and tested by a qualified clinical laboratory technician using sterilized needles, slides and chemicals like anti-A, anti-B, anti-D.

3.4. Methods of Data Analysis

The genetic structure of the population is determined by the total of all alleles (the gene pool). The genetic structure can be described in terms of allelic and genotypic frequencies (Russell, 2005). Allele frequencies were calculated under the assumption of Hardy–Weinberg equilibrium by using Excel software and SPSS software version 20.

The frequencies of the genotype at equilibrium were computed by the square of the allelic frequencies $(p + q + r)^2 = p^2$ (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO) (Griffith *et al.,* 2008). For this study the allelic frequencies were determined using the Bernstein method as follows (Nam & Gart, 1976). If p, q and r are A, B and O respectively,

$$p = 1 - \sqrt{freq(B) + freq(O)},$$

$$q = 1 - \sqrt{freq(A) + freq(O)} \text{ and}$$

$$r = \sqrt{freq(O)}$$

If $p + q + r \neq 1$, a correction factor must be carried out by the deviation $D = 1 - (p + q + r)$. So, the frequencies of blood group alleles are adjusted as follows to obtain the final frequencies p', q' and r'.

$$p' = p\,(1+D/2),$$
$$q' = q\,(1+D/2) \text{ and}$$
$$r' = (r+D/2)\,(1+D/2)$$

Chi-square test was used to compare observed phenotype frequency distribution of ABO and Rh (D) blood groups to that expected under the Hardy-Weinberg law. Pearson chi-square goodness of fit test statistic is: $\chi^2 = (O-E)^2/E$ where O are observed count and E are corresponding expected count which are calculated by $p^2 + 2pr$, $q^2 + 2qr$ and r^2 formula for A, B, and O respectively.

4. RESULTS AND DISCUSSIONS

4.1. Distribution of Blood Groups Phenotypes among Students

4.1.1. Distribution of ABO blood group phenotypes among students of three ethnic groups

For this study, a total of 384 students were purposively selected from among a total of 130 females and 254 males. The distribution of ABO and Rh (D) blood groups among three ethnic groups is represented in Table 6 and 7.

Table 7. Distributions of ABO blood group phenotype among students of three ethnic groups

Ethnic Group	Sex	Distribution of ABO Blood Groups				Total (%)
		A (%)	B (%)	AB (%)	O (%)	
Sodo Oromo	Male	44(24.72)	32(17.98)	9(5.06)	34(19.10)	119(66.85)
	Female	20(11.24)	12(6.74)	3(1.69)	24(13.48)	59(33.15)
	Subtotal	64(35.96)	44(24.72)	12(6.74)	58(32.58)	178(100)
Walane	Male	26(25.49)	14(13.73)	8(7.84)	21(20.59)	69(67.65)
	Female	4(3.92)	9(8.82)	4(3.92)	16(15.69)	33(32.35)
	Subtotal	30(29.41)	23(22.55)	12(11.76)	37(36.27)	102(100)
Kistane	Male	19(18.27)	20(19.23)	4(3.85)	23(22.12)	66(63.46)
	Female	4(3.85)	18(17.31)	2(1.92)	14(13.46)	38(36.54)
	Subtotal	23(22.12)	38(36.54)	6(5.77)	37(35.58)	104(100)
	Total male	89(35.04)	66(25.98)	21(8.27)	78(30.71)	254(100)
	Total female	28(21.54)	39(30)	9(6.9)	54(41.54)	130(100)
Total		117(30.47)	105(24.34)	30(7.81)	132(34.38)	384(100)

There were differences in frequency distribution of the blood group (ABO) among students' of Sodo Oromo, Walane and Kistane in the study. The different blood types and distribution of ABO blood group system was recorded in the three groups (Tables 6). The most prevalent blood group was type O followed by A, B, and AB (Table 6). Blood group O was more frequent in the Walane than Kistane and Sodo Oromo ethnic

groups. The frequency of blood group A was higher in Sodo Oromo than Walane and Kistane ethnic groups and blood group B is dominant in the Kistane ethnic group than in Sodo Oromo and Walane. Blood group AB was more frequent in Walane than in Sodo oromo and Kistane ethnic groups (Table 6).

Of the 178 blood samples belonging to the Sodo Oromo, the A blood type was more frequent in males while O blood type was highly distributed in females, but AB blood type was the least frequent in both sexes in Sodo Oromo ethnic group. Of the 102 blood samples of Walane, A blood type was highly distributed in males while O blood type was highly frequent in females, but AB blood type was the least frequent in both sexes in Walane ethnic group. Also of the 104 blood samples belonging to the Kistane, the O blood type was highly frequent in males while B blood type was highly frequent in females, but AB blood type was the least frequent in both sexes in Kistane ethnic group (Table 6).

As indicated in Table 6, A blood type was more frequent in males while AB blood type was least frequent. O blood type was more frequent than that of AB blood type in females. From this study the O blood type was more frequent in females while the A blood type was more frequent in males in whole population.

Different studies have shown (Table 4) that blood group O was the most common blood group and blood group AB was the least common blood group in different ethnic groups (Bakare *et al*., 2006). Similarly an Egyptian population, O is, 33%, A is, 30%, B is 24%, AB 8% (Hamed *et al*., 2012) and Akinnuga *et al*. (2011) had reported that students were 27% A blood type, 16% B blood type, 4% AB blood type and 53% O blood type in Nigerian population which is in agreement with this study. However, the finding of this study seem to deviate from the result obtained by Lee and his colleagues on the ABO and Rh (D) blood groups and gestational hypertensive disorders in Kenyan population when ABO blood group distribution is O>B>A>AB (Lee *et al*., 2012). It also does not agree with the results obtained in Sudan where the distribution of O 62%; A, 16%; B, 21% and AB, 0% (Tagny *et al*., 2009a), was reported.

4.1.2. Distribution of Rh (D) blood group phenotypes among students of the three ethnic groups

Table 8. Phenotypic distribution of Rh (D) blood groups among students of the three ethnic groups

Ethnic groups	Distribution of Rh (D) Blood Groups		Total
	Rh^+ (%)	Rh^- (%)	(%)
Sodo Oromo	174 (97.75)	4 (2.25)	178 (100)
Walane	98 (96.08)	4 (3.92)	102 (100)
Kistane	98 (94.23)	6 (5.77)	104 (100)
Total	370 (96.35)	14 (3.65)	384 (100)

The variations in the frequency distribution of Rh^+ and Rh^- among the three ethnic groups followed the same pattern as shown in Table 7. The percentage distribution of Rh (D) blood groups among the three groups was 96.35% and 3.65% Rh^+ and Rh^- respectively (Table 7). The Rh^+ blood groups were the most predominant; but Rh^- blood groups were the least distributed in the population.

4.2. Distribution of ABO Blood Group Phenotypes Based on Rh (D) Blood Groups

The percentage distribution of the ABO blood groups is based on the Rh (D) blood groups (Table 8). The ABO blood group distribution is based on Rh (D) in Sodo Oromo; blood group A with Rh^+ is 34.83% while in Walane ethnic group blood group A with Rh^+ is 29.41% but in Kistane the percentage of blood group A is reduced to 20.19% of the total population. As it is indicated in Table 8, blood group AB with Rh positive has a small percentage distribution in the three ethnic groups than blood group A and B. Blood group O with Rh positive of Sodo Oromo was 32.58% and that of the Walane was 34.32% which is higher than the Sodo Oromo ethnic group and 31.73% for the Kistane ethnic group. So, blood group O with Rh positive is dominant in the Walane ethnic group. As compared to the other blood groups, blood group O with Rh positive percentage distribution varies significantly ($P<0.05$) in the three ethnic groups. However, the percentage distribution of Rh negative is very rare in the three ethnic groups.

Table 9. Co- distribution of ABO and Rh (D) blood groups among three ethnic groups of current study

Ethnic Groups	Rh(D) Blood Groups	ABO Blood Groups				Total (%)
		A (%)	B (%)	AB (%)	O (%)	
Sodo Oromo	Positive	62(34.83)	44(24.72)	10(5.62)	58(32.58)	174(97.75)
	Negative	2(1.12)	-	2(1.12)	-	4(2.25)
Walane	Positive	30(29.41)	21(20.59)	12(11.76)	35(34.32)	98(96.08)
	Negative	-	2(1.96)	-	2(1.96)	4(3.92)
Kistane	Positive	21(20.19)	38(36.54)	6(5.77)	33(31.73)	98(94.23)
	Negative	2(1.92)	-	-	4(3.85)	6(5.77)
Total	Positive	113(30.54)	103(27.84)	28(7.57)	126(34.05)	370(96.35)
	Negative	4(3.04)	2(1.96)	2(1.12)	6(5.81)	14(11.93)
						384(100)

4.3. Estimation of the Genotypic and the Allelic Frequencies

4.3.1. Estimation of ABO and Rh (D) blood group genotype and allelic frequencies among the students of three ethnic groups

The main application of the Hardy-Weinberg law is to estimate the heterozygous and homozygous distribution in population study. The genotypic and allelic frequency distribution among the students of three ethnic groups was calculated and the results are shown in Table 9.

Table 10. Estimation of the genotypic and the allelic frequencies among three ethnic groups

Ethnic group	Allele	Frequency	Genotype	Frequency	Phenotype	Frequency (%)
Sodo Oromo	O(r)	0.5708	I^OI^O	0.3258	O	32.58
	A(p)	0.2571	I^AI^A	0.0661	A	6.61
	B(q)	0.1721	I^AI^O	0.2935	A	29.35
			I^BI^B	0.0296	B	2.96
			I^BI^O	0.1965	B	19.65
			I^AI^B	0.0885	AB	8.85
	D	0.8507	DD	0.7237	Rh(D) +ve	72.37
	d	0.1493	Dd	0.2540	Rh(D) +ve	25.4
			dd	0.0223	Rh(D) -ve	2.23
Walane	O(r)	0.6023	I^OI^O	0.3628	O	36.28
	A(p)	0.2331	I^AI^A	0.0543	A	5.43
	B(q)	0.1646	I^AI^O	0.2808	A	28.08
			I^BI^B	0.0271	B	2.71
			I^BI^O	0.1983	B	19.83
			I^AI^B	0.0767	AB	7.67
	D	0.8020	DD	0.6432	Rh(D) +ve	64.32
			Dd	0.3176	Rh(D) +ve	31.76
	d	0.1980	dd	0.0392	Rh(D) -ve	3.92
Kistane	O(r)	0.5965	I^OI^O	0.3558	O	35.58
	A(p)	0.1631	I^AI^A	0.0266	A	2.66
	B(q)	0.2404	I^AI^O	0.1946	A	19.46
			I^BI^B	0.0578	B	5.78
			I^BI^O	0.2868	B	28.68
			I^AI^B	0.0784	AB	7.84
	D	0.7598	DD	0.5773	Rh(D) +ve	57.73
			Dd	0.3650	Rh(D) +ve	36.5
	d	0.2402	dd	0.0577	Rh(D) -ve	5.77

As indicated in Table 9, most of A and B blood types are heterozygous in each of the ethnic groups. The genotype of I^AI^O makesup 0.2935, 0.2808 and 0.1946 in Sodo Oromo, Walane and Kistane, respectively. While I^BI^O makesup 0.1965, 0.1983 and 0.2868 in Sodo Oromo, Walane and Kistane, respectively. The results of this study agree with the suggestion of Bakare *et al.* (2006) that the predominance of O allele may also be as a result of the fact that many A's and B's may have been heterozygous carrying O allele

silently thereby maintaining O allele in the heterozygous population. Homozygous blood group A of the three ethnic groups was calculated to be 6.61%, 5.43% and 2.66% in Sodo Oromo, Walane and Kistane, respectively.

This indicates that genotype I^AI^A was more frequent in Sodo Oromo than in the two ethnic groups and the least in Kistane. Whereas the homozygous blood group B obtained for the three ethnic groups was 2.96%, 2.71% and 5.78% in Sodo Oromo, Walane and Kistane respectively. Homozygous blood group B is the highest frequent in Kistane and the least in Walane.

In the case of Rh (D) blood grouping system, of the 384 sample population 96.35% of population sampled were Rh^+ and 3.65% Rh^-. As indicated in Table.9, the heterozygous Dd positive was 0.254, 0.3176 and 0.365 in Sodo Oromo, Walane and Kistane respectively. This result shows that heterozygous genotype Dd is the highest in Kistane while the least in Sodo Oromo. Homozygous DD positive genotype of the three ethnic group calculated by using Hardy-Weinberg equilibrium were 0.7237 in Sodo Oromo, 0.6432 in Walane and 0.5773 in Kistane. So, the homozygous DD positive is more distributed in Sodo oromo than in the others two ethnic groups; whereas least distributed in Kistane. Also the homozygous recessive genotype dd negative was calculated as 2.23% in Sodo Oromo, 3.92% in Walane and 5.77% in Kistane which show homozygous genotype dd negative is the highest frequent among the Kistane than two ethnic group while least in Sodo Oromo.

The overall allelic frequencies of the population as it was calculated using the extension of the Hardy–Weinberg law were 0.59, 0.21 and 0.20 for I^O, I^A and I^B alleles, respectively. Also on the Rhesus status, the allelic frequencies were 0.81 and 0.19 for D and d alleles, respectively (Table 9).

4.3.2. The chi-square test for each ethnic group

The chi- square test for each ethnic group was calculated by using the result obtained from Table. 9 and the ABO and Rh (D) frequency distribution to know whether the population of each ethnic group is in Hardy- Weinberg equilibrium or not.

4.3.3. Chi-square test in ABO blood group

In this study the observed ABO blood group distribution from each ethnic group was compared under Hardy- Weinberg equilibrium with the expected value.

Table 11. Chi-square test for Sodo Oromo in ABO blood group

Blood group	Observed No (O)	Expected No (E)	Difference D = (O-E)	D^2	D^2/E
O	58	57.9924	0.0076	5.8×10^{-5}	9.96×10^{-7}
A	64	64.0088	-0.0088	7.7×10^{-5}	1.3×10^{-6}
B	44	40.2458	3.7542	14.0940	0.3502
AB	12	15.7530	-3.753	14.0850	0.8941
Total	178	178			$\chi^2 = 1.2443$

As indicated in Table 10, calculated chi-square is 1.2443 which have the $p < 0.05$ with 3 degree freedom. This means that there is no significant difference between observed and expected in Sodo Oromo ethnic group.

Table 12. Chi-square test for Walane in ABO blood group

Blood group	Observed No (O)	Expected No (E)	Difference D = (O-E)	D^2	D^2/E
O	37	37.0056	0.0056	3.14×10^{-5}	8.47×10^{-7}
A	30	34.1802	-4.1802	17.4741	0.5112
B	23	22.9908	0.0092	8.46×10^{-5}	3.68×10^{-6}
AB	12	7.8234	4.1766	17.444	2.2297
Total	102	102			$\chi^2 = 2.741$

In Walane ethnic group the chi-square value obtained 2.741, the $p < 0.05$ with degree freedom 3, the result shows that the ABO blood group distribution between observed and expected in Walane ethnic group did not show significant difference.

Table 13. Chi-square test for Kistane in ABO blood group

Blood group	Observed No (O)	Expected No (E)	Difference D = (O-E)	D^2	D^2/E
O	37	37.0032	-0.0032	1.02×10^{-5}	2.8×10^{-7}
A	23	23.0048	-0.0048	2.3×10^{-5}	1×10^{-6}
B	38	35.8384	2.1616	4.6725	0.1304
AB	6	8.1536	-2.1536	4.638	0.5688
Total	104	104			$\chi^2 = 0.6992$

Finally, the chi-square test analysis for Kistane was calculated to be 0.6992, the $p < 0.05$ with 3 degree of freedom. This result indicates that the ABO blood group distribution in Kistane shows no significant difference.

4.3.4. The chi- square test in Rh (D) blood group

The chi-square test for three ethnic groups are compared with observed and expected number whether the population in each ethnic group in Hardy- Weinberg equilibrium or not.

Table 14. Chi-square test for Sodo Oromo in Rh (D) blood group

Blood group	Observed No (O)	Expected No (E)	Difference D = (O-E)	D^2	D^2/E
Rh^+	174	174.0306	-0.0306	9.4×10^{-4}	5.4×10^{-6}
Rh^-	4	3.9694	0.0306	9.4×10^{-4}	2.4×10^{-4}
Total	178	178			$\chi^2 = 0.0002$

The calculated chi-square in Sodo Oromo is 0.0002, which has the $p > 0.05$ with 1 degree freedom. This means that there is statistically insignificant difference between observed and expected value in Sodo Oromo.

Table 15. Chi-square test for Walane in Rh (D) blood group

Blood group	Observed No (O)	Expected No (E)	Difference D = (O-E)	D^2	D^2/E
Rh^+	98	98.0016	-0.0016	2.56×10^{-6}	2.612×10^{-8}
Rh^-	4	3.9984	0.0016	2.56×10^{-6}	6.4×10^{-7}
Total	102	102			$\chi^2 = 6.7\times10^{-7}$

In Walane the chi- square is 6.7×10^{-7} which has the $p > 0.05$ with 1 degree freedom. The Walane ethnic group showed statistically insignificant difference between the observed and the expected.

Table 16. Chi-square test for Kistane in Rh (D) blood group

Blood group	Observed No (O)	Expected No (E)	Difference D = (O-E)	D^2	D^2/E
Rh^+	98	97.9992	0.0008	6.4×10^{-7}	6.5×10^{-10}
Rh^-	6	6.0008	-0.0008	6.4×10^{-7}	1.06×10^{-10}
Total	104	104			$\chi^2 = 1.07\times10^{-7}$

Finally, the chi- square test for Kistane ethnic group is 1.07×10^{-7} which has the $p> 0.05$ with 1 degree freedom. The Kistane ethnic group shows statistically insignificant difference between the observed and the expected.

.

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

The ABO and Rh (D) blood groups are hereditary characters and are useful in population genetic studies. There are many blood group systems on the basis of different blood group antigens, but only ABO and Rh (D) blood groups are the most important in clinical practice. The study was carried out in Harbu Chulule Preparatory and Secondary School at Saden Sodo District, South West Shewa Zone, Oromia Regional State, Ethiopia. Sodo Oromo population size is 74,338, Walane is 9,175 and that of Kistane population 9,613 out of the total population district.

The study was conducted on 384 students comprising approximately 46% of the student population. Individuals were purposively selected and the information about the ethnic group was provided by students themselves when filling their personal profile form, before conducting blood test. Therefore, ethnicity was self-declared and stratified along ethnic lines. Blood sample was taken from finger prick after wiping the finger with a piece of cotton saturated with 70% alcohol and piercing it with a sterile disposable lancet.

The blood samples were collected and tested by a qualified clinical laboratory technician using sterilized needles, slides and chemicals like anti-A, anti-B, anti-D. Allele frequencies were calculated under the assumption of Hardy–Weinberg equilibrium by using Excel software and SPSS software version 20. The Percentage distribution of the ABO blood group among the three ethnic groups were 30.47%, 27.34%, 7.81% and 34.38% for blood group A, B, AB and O, respectively, 96.35% and 3.65% Rh^+ and Rh^- in the population of Saden Sodo district respectively.

5.2. Conclusion

The distribution of ABO blood group varies among students of Sodo Oromo, Walane and Kistane, which blood type A was more frequent in Sodo Oromo followed by O, B and AB, respectively, while O blood type was more frequent in Walane, followed by A, B, and AB. But in Kistane B blood type was more frequent followed by O, A, and AB respectively. In case of Rh (D) blood groups 97.75% Rh^+ and 2.25% Rh^- in Sodo Oromo, 96.08% Rh^+ and 3.92% Rh^- in Walane and 94.23% Rh^+ and 5.77% Rh^- in Kistane respectively.

The overall allelic frequencies of the population were 0.59, 0.21 and 0.20 for I^O, I^A and I^B alleles, respectively. Also on the Rhesus status, the allelic frequencies were 0.81 and 0.19 for D and d alleles, respectively. From this study I conclude that no significance difference between among each ethnic groups.

So, I believe that data from this study have provided information on the genetic variability and polymorphism of the blood group and rhesus antigens among the population in Saden Sodo District. This information would be useful to the geneticists and to the clinicians especially in the planning of blood transfusion programs since they play integral role of the genetic profile of the Saden Sodo population.

5.3. Recommendation

Based on the result of this study, the following can be recommended:

- From this study A blood type was more frequent followed by O, B, AB in Sodo Oromo, B blood type was more frequent in Kistane which not agree with worldwide study; so, further study may need in the Saden Sodo district populations too even in the country.
- For this study there was no B^- and O^- in Sodo Oromo, A^- and AB^- in Walane and B^- and AB^- in Kistane; the sample size may not represent the three ethnic groups, further study with more sample size is needed.
- From this study O>A>B>AB in Saden Sodo population. So, further study was recommended at Saden Sodo district with large sample size, even in South West Shewa Zone.

6. REFERENCES

Adeyemo OA, Soboyejo OB. 2006. Frequency distributions of ABO, RH blood groups and blood genotypes among the cell biology and genetics students of University of Lagos, Nigeria. *Afr J Biotechnol*, 5(22): 2062-2065.

Akabas, F., M. Aydin and Cenani. 2003. ABO sub blood group allele frequencies in the Turkish population. *Anthropol. Anz*. 61:257-60.

Akinnuga BO, Amosu AM, Ugwah GU. 2011. Distribution of ABO and Rh Blood Groups among Major Ethnic Groups of Medical Students of Madonna University Teaching Hospital, Elele, Nigeria. *Asian Journal of Medical Sciences*, 3(3): 106-109.

Alimba CG, Adekoya KO, Oboh BO. 2010. Prevalence and gene frequencies of phenylthiocarbamide (PTC) taste sensitivity, ABO and Rhesus factor (Rh) blood groups, and hemoglobin Variants among a Nigerian population. *Egypt J Med Hum Genet*; 11:153–8.

Anees M, Shabir Mirza M. 2005.Distribution of ABO and Rh blood group alleles in Gujrat region of Punjab, Pakistan. *Proc Pakistan Acad Sci*; 42(4):233–8.

Anstee, D.J. 2009. Red blood cell genotyping and the feature of parents fusion testing. *Blood* 114 (2):248-56.

Avent, N.D. 2009. Large-scale blood group genotyping: clinical implication. *Br J Heamatoll* 44 (1):3.

Bakare A.A, Azeez MA, and Agbolade JO. 2005. Gene frequencies of ABO and Rhesus blood groups and hemoglobin variants in Ogbomoso, south-west Nigeria. *Afr J Biotechnol*, 5(3): 224-229.

Bakare, A., A.M., A. Azeez. and J.O. Agbolade. 2006. Gene frequencies ABO and Rhesus blood groups and hemoglobin variants in Ogbomosho, south east Nigeria. *Afr. J. Biotechnol*. 5:224-229.

Bloodbook.com. Retrieved. 2010. Racial and ethnic distribution of ABO blood types. 08-01

Bruce, M.G, 2002. BCF-Members chairman's annual report. 2001. *The blood care foundation.* http://www. Blood care. Org. uk/html/resources chairman. Accessed on June.

Chou S.T., and West Hoff CM. 2010. The Rh and Rh AG blood group systems. *Immunohematology*; 26(4):178–86.

Chown, B. Lewis and K. Kaita. 1957. A new cell blood group phenotype. *Nature*180 (4588); 711.

Daniel, G. 2002. *Human Blood Groups*, *2nd Edition*. Blackwell Science.

Daniel, G. 2005. The molecular genetics of blood group polymorphism. *Transplant immune*. 14 (3-4):143-153.

Daniel, H.L and A.G., clark. 2007. *Principle of population genetics, 4th Edition*. Siauer. Associates. Sunderland, Massachusetts. P633.

Das, P.K., Nair, S.C., Harris, V.K., Rose, D., Mammen, J.J., Bose, Y.N. and Sudarsanam, A. 2001. Distribution of ABO and Rh-D blood groups among blood donors in a tertiary care centre in South India. *Tropical Doctor*, 31, 47.

Ember, C.R., Ember, M.R. and Peregrine, P.N. 2010. *Anthropology*, *13th Edition*. Pearson, New Jersey.

Enosolease ME, and Bazuaye GN .2008. Distribution of ABO and Rh-D blood groups in the Benin area of Niger-Delta: Implication for regional blood transfusion. *Asian J TransfusSci* 2: 3-5.

Fareed, Mohd; Hussain, Ruqaiy; Shah, Ahsana Afzal, and Muhammad. 2014. Frequencies among six population of Jammu and Kashmir, India. *Transfusion and Apheresis science* 50(2)247.

Gannon, WF. 1995. *Reviews of Medical Physiology, 17th Edition*. Practical Hall International Inc. London: pp 487-9.

Garratty, G., W. Dzink. PD. Issitt, D. M., Lublin Reid. and T. Zelinski. 2000. Termology for blood group antigens and genes-historical origins and guide line in the new millennium. *Transfusion*; 40; 477-489.

Garratty, G., Glynn, S.A. and McEntire, R. 2004. ABO and Rh (D) phenotype frequencies of different racial ethnic groups in the United States. *Transfusion*, 44, 703.

Greer, JB., Yazer MH, Raval JS, Barmada MM, Brand RE, and Whitcomb DC. 2010. Significant association between ABO blood group and pancreatic cancer. *World J Gastroenterol*; 16(44): 5588–91 (Retrieved from <http://www.wjgnet.com/1007-9327/full/v16/i44/5588.htm>).

Green, D.O., Jarret. K. J. Ruth, A.R. Folson, K. Liu. 1995. Relationship among Lewis phenotype, clotting factors and others cardiovascular risk factors in young adults. *Lab Clin Med*. 125:334-9.

Greenwalt, T.J.1997. A short history of transfusion medicine. *Transfusion*; 37(5):550=5 63.

Griffthis, A.F.S.R., wessler, R.C. Lewontin and S.B. Carroll. 2008. *Introduction to Genetic Analysis, 9th Edition*. W. H. Freeman and company. New york.p838.

Hamed, C.T., Bollahi, M.A., Abdelhamid, I., Med Mahmoud, M.A., Ba, B., Ghaber, S., Habti, N. & Houmeida, A. (2012). Frequencies and ethnic distribution of ABO and Rh (D) blood groups in Mauritania: results of first nationwide study. *International Journal of Immunogenetics*, 39, 151.

Hasna, A. M. 2010. A study of ABO blood group and Rh factor distribution. *Journal of Basrah Researches (Science) vol*. (36) A /No (5). 15.

ISBT (International Society of Blood Transfusion) URL accessed on 2006. 11-14.

Khalil, I.A., Phrykian S, Farr AD.1989. Blood group distribution in Sudan. Gene Geogr; 3:7–10.

Khattak ID, Khan TM, Syed P, Shah AM, Khattak ST, Ali A.2008. *Frequency of ABO and Rhesus blood groups in district Swat*, Pakistan. *J Ayub Med Coll Abbottabad*; 20 (4):127–9.

Khurshid, B., M. Naz, M. Hassen, S.F. Mabood. 1992. Frequency of ABO and Rh (D) blood groups in direct Swabi N.W.F.P (Pakistan). *J.Sc. Tech Univ. Peshwar*: 16; 5-6.

Klug WS, and Cummings MR. 2002. *Essentials of Genetics, 4th Edition*. New Jersey, USA: Prentice Hall; p508.

Landsteiner, K.1900. Zur Kenntnisder anti-fermenativen, Lytischen and aggutinierenden Wirkungen des Blutserumas underlymph. *Zentral blatt Bacteriologie*. 27:357-62.

Landsteiner, K., Weiner, A.S. 1940. An agglutinable factor in human blood recognize by immune sera for rhesus blood. *Proc. Sco. Biol. Med*. 43:223-224.

Lee, B.K., Zhang, Z., Wikman, A., Lindgvist, P.G. & Reilly, M. 2012. ABO and RhD blood groups and gestational hypertensive disorders: a population-based cohort study. *BJOG*, 119, 1232.

Letsky, I., J. M. Leck and Bowman. 2000. *Rhesus and other Hemolytic Disease. Antenatal and Neonatal Screening, 2nd Edition*. Oxford University Press. ISBN978-0-19-262,826-8.

Loua, A., Lamah, MR. Haba NY, Camara M. 2007. Frequency of ABO blood group and rhesus D in the Madagascar population. *Transfus Clin Biol*; 14:435–9.

Lyko, J., Gaertner H, Kaviti JN, Karithi MW, and Akoto, B. 1992. The blood group antigens ABO and Rh in Kenyans. *Hamdard Medicus*; 35:59–67.

Masushita, S., Timmamura, T. Mizuta and M. Hanada. 1983. Acquired b antigen and poly agglutination in the patient with gastricancer. *The Japanese Journal of Surgery* 13(6); 540-2.

Maton, Anthea, Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon Warner, David LaHart, and Jill, D. Wright .1993. Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall. ISBN 0-13-981176-1

Mayo, O, 2008. A century of Hardy-Weinberg Equilibrium. *Twin Research and Human Genetics* 11(3):249-256.

Muhammad S, Jeff. 2010. ABO and rhesus blood group distribution in Kurds. 2010. *Journal of Blood Medicine*: volume 143-146.

Millision, P.L. 1994.The genetic bases of the Rh blood group system. *Transfusion*; 34; 539-541.

Nam, J.M. and Gart, J.J. 1976. Bernstein's and gene counting methods in generalized ABO like systems. *Annals of Human Genetics*, 39, 361.

Nickel, R.G., Fiedshoff and L.Robert.1999. Determination of Duffy genotypes in three population of America descent using PCR and sequence of specific oligo-nucleotides. *Human immunology* 60(8):738-42.

Novak, P.O.1995. *Dorland Pocket Medical Dictionary, 25th Edition*. W.B. Saunders Company, Pennsylvania. 351-75.

Office of Land management and Environmental Conservation of Saden Sodo District 2011.

Office of Land management and Environmental Conservation of Saden Sodo District 2015.

Povey, S.C., A. Slaugher, D.F. Wilson, I. P. Gormly, K. E. Bukton, P. A. Perry and M.Bobrow.1978. Evidence for the assignment of loci AK 3. And AconS to chromosome 9 in man. *Ann Hums Genet* 38:1-5.

Reid, M.E. and C., Lomas-Francis. 2004. *The Blood Group Antigen Facts Books, 2nd Edition*. New York: Elsevier Academic Press.

Russell, J. P. 2005. *Genetics Mendelian Approach*. Pearson Benjamin Cummings. New York p 842.

Saladin, K. 2003. *Anatomy and Physiology: The Unity of Form and Function, 3rd Edition*. The McGraw-Hill Companies, USA, Pp. 679-698.

Seely, RR. Stephans, TD. Tate, P.1998. *Anatomy and Physiology*, *4th Edition*. The McGraw Hill Companies, Inc. USA. P1098.

Simpkins, J and Williams, J. I. 1997. *Advanced Biology*, *3rd Edition.* Collins Education, London. P760.

Srikumari, C. R., J. Rajanikumari and Rao, T.V. 1987. Acuity of selective mechanisms operating on ABO. Rh (D) and MN blood groups. *Am.J.Phy.Anthrop*.72 (1):117-121.

Tagny, C.T., Diarra, A., Yahaya, R., Hakizima, M., Nguessan, A. and Mensa, G. (2009a). Characteristics of blood donors and donated blood in sub-Saharan Francophone Africa. *Transfusion*, 49, 1592.

Tuaszik, T. 1995. Heterogeneity in the distribution of ABO blood groups in Hungary. *Gene Geogr*; 9: 169-76.

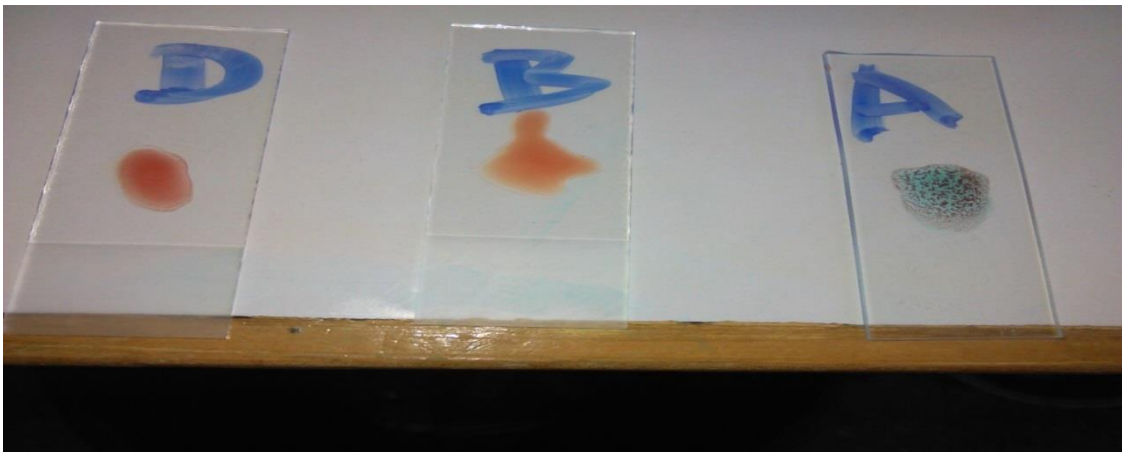
Urbniac, S.J. and Graiss, M.A. 2000. Rh hemolytic disease of the fetus and the newborn. *Blood Rev*.14:44-61.

Von Decastella A, and Sturli, A. 1902.Ureber dies isoagglutinine in serumgesunder and Kranaker Menschen. *Mfiner Med WSchr*; 49:1090–5.

APPENDIX FIGURE

Appendix figure 1. While on the process of taking blood sample taken from voluntary students

A. A Positive Group



C **B** **A**

Appendix figure 2. A positive blood groups

Figures 2 (A, B, C) shows the A Positive blood group sample on adding Antigen-A, Antigen-B and Antigen D respectively. When Antibody A, Antibody B and Antibody D was added on the positive A blood type, the blood sample on slide A and C was form aggregates (agglutinate) but the blood sample found on slide B remain fluid (No aggregates).

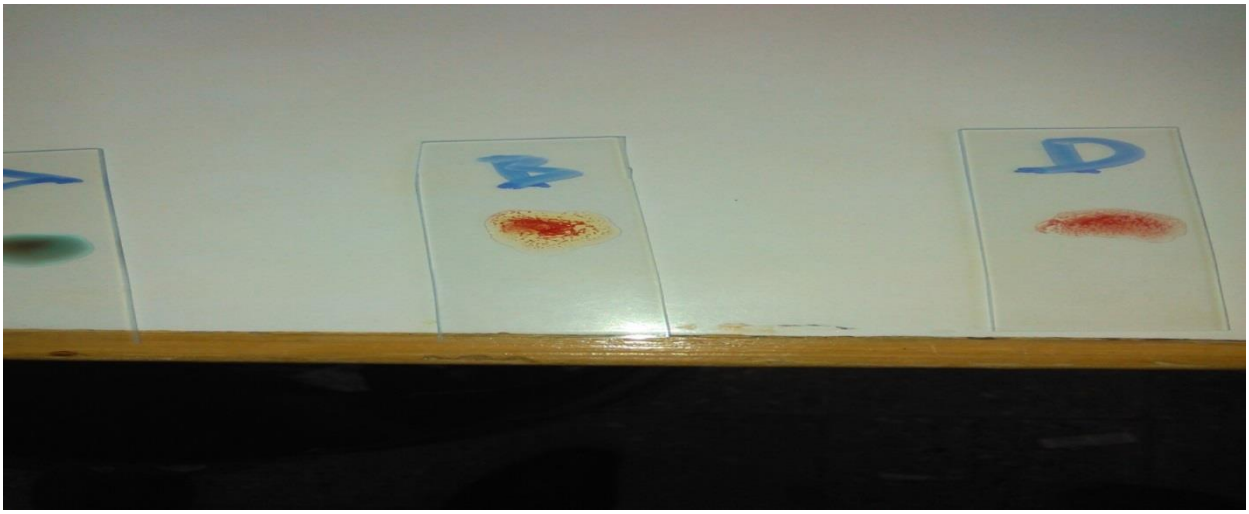
B. A negative group

A **B** **C**

Appendix figure 3. A negative blood groups

Figure 3 (A, B, C) shows the A Negative blood group sample on adding Antigen-A, Antigen-B and antigen-D respectively. When Antibody A, Antibody B and Antibody D was added on the positive A blood type, the blood sample on slide A was form clump (agglutinates) but the two blood sample found on slide B and C remain fluid (No clump)

C. B Positive Group



A **B** **C**

Appendix figure 4. B positive blood groups

Figure 4 (A, B, C) shows the B Positive blood group sample on adding Antigen-A, Antigen-B and Antigen-D respectively. When Antibody A, Antibody B and Antibody D was added on the positive B blood type, the blood sample on slide B and C was form aggregates (agglutinates) but the blood sample found on slide A remain fluid (No aggregates).

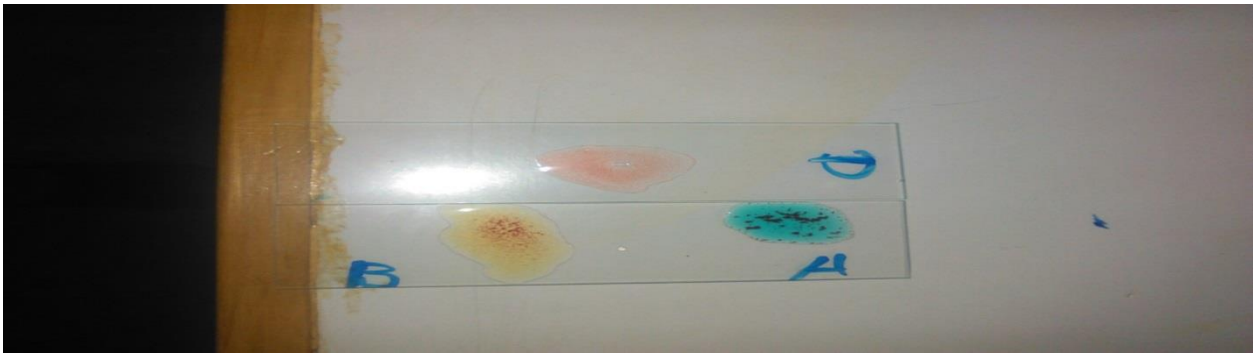
D.B Negative Group

A **B** **C**

Appendix figure 5. B negative blood groups

Figure 5 (A, B, C) shows the B Negative blood group sample on adding Antigen-A, Antigen-B and Antigen-D respectively. When Antibody A, Antibody B and Antibody D was added on the positive B blood type, the blood sample only on slide B was form aggregates (agglutinates) but the two blood sample found on slide A and C remain fluid (No aggregates).

E. AB Positive Group



B **A** **C**

Appendix figure 6 . AB positive blood groups

Figure 6 (A, B, C) shows the AB Positive blood group sample on adding Antigen-A, Antigen-B and Antigen-D respectively. When Antibody A, Antibody B and Antibody D was added on the positive AB blood type, all blood sample found on slide A, B and C was form aggregates (agglutinates).

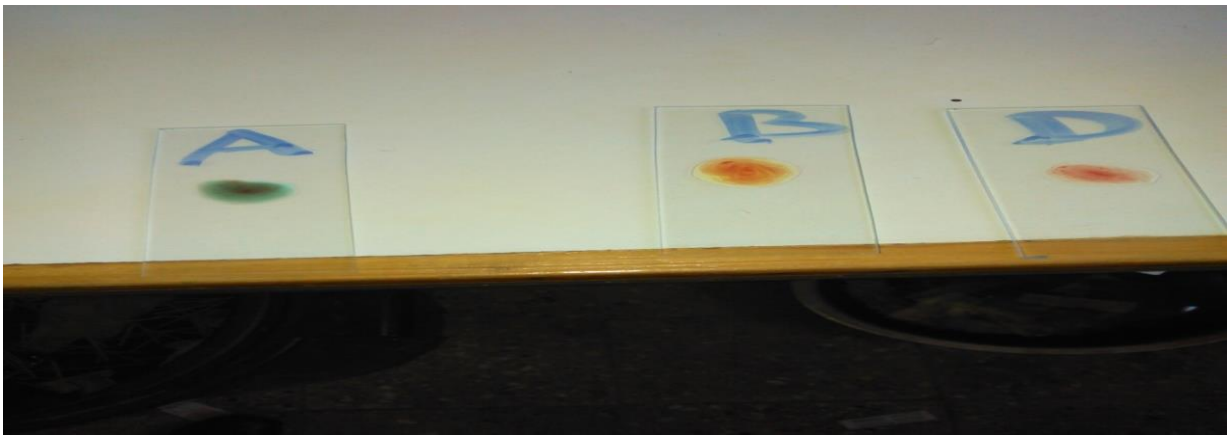
F. AB Negative Group

A **B** **C**

Appendix figure 7 . AB negative blood groups

Figure 7 (A, B, C) shows the AB Negative blood group sample on adding Antigen-A, Antigen-B and Antigen-D respectively. When Antibody A, Antibody B and Antibody D was added on the negative AB blood type, the blood sample on slide A and B was form aggregates (agglutinates) but the blood sample found on slide C remain fluid (No aggregates).

G.O Positive Group



A **B** **C**

Appendix figure 8. O positive blood groups

Figure 8 (A, B, C) shows the O Positive blood group sample on adding Antigen-A, Antigen-B and Antigen-D respectively. When Antibody A, Antibody B and Antibody D was added on the positive O blood type, the blood sample found only on slide C was form aggregates (agglutinates) but the two blood sample found on slide A and B remain fluid (No aggregates).

H. O Negative Group

C **B** **A**

Appendix figure 9. O negative blood groups

Figure 9 (A, B, C) shows the O Negative blood group sample on adding Antigen-A, Antigen-B and Antigen-D respectively. When Antibody A, Antibody B and Antibody D was added on the negative O blood type, all blood sample found on slide A, B and C was remain fluid (No aggregates).