FREQUENCIES OF ABO AND Rh(D) BLOOD GROUP ALLELES AMONG STUDENTS OF BORA SECONDARY SCHOOL, BORA WOREDA, EAST SHOA ZONE, OROMIA REGION, ETHIOPIA

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POST GRADUATE PROGRAM DIRECTORATE

We hereby certify that We have read and evaluated this thesis entitled "Frequencies of ABO and Rh (D) Blood Group Alleles among Students of Bora Secondary School, Bora Woreda, East Shoa Zone,Oromia Region, Ethiopia."

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DEDICATION

I dedicate this piece of Research work to my beloved parents, Abeje kebebe and Bezunesh Yifru, who gave me life, and the best possible provisions for my life, better than anyone can ever get!!

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 compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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BIOGRAPHICAL SKETCH

The author was born on 20 June 1979, from his father Abeje Kebebe and from his mother Bezunesh Yifru at Meseranje Koshore Kebele, Robe District, Arsi Zone, Oromia Regional State. He attended his elementary education at Meseranje Abu School and junior Education at Robe Junior Secondary School from 1983-1990. After successfully passing his grade eight exam, he studied at Didea Senior Secondary School from 1991 -1995. In 1998 he joined Haramaya University and attended his degree program in Biology. The author worked as a high School teacher teaching Biology in different schools until he joined the School of Graduate Studies of Haramaya University to study Master of Science in Biology in July 2013 sponsored by the Ministry of Education.

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ACRONYMS AND ABBEREVIATIONS

CSA	Central Statistical Agency
MOE	Ministry of Education
FMC	Fresenius Medical Care
HDN	Hemolytic Disease of the Newborn
HWP	Hardy-Weinberg principle
IgM	Immune gamma globulin
ISBT	International Society of Blood Transfusion
PCR	Polymerase Chain Reaction
RBCs	Red Blood Cells
Rh	Rhesus
SPSS	Statistical package for Social Sciences

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ABSTRACT

ABO and Rh blood groups vary worldwide and are not distributed uniformly even among related ethnic groups. The study was aimed at having information on the allelic frequency, genotypic frequency of ABO and Rh blood group phenotypes among the students of Bora Secondary School. From a total of 719 students 240 voluntary participant students were `selected for this study. Among them, 134 (55.83%) were males and 106(44.17%) were females. Blood samples were collected by pricking the middle finger and a drop of each antisera; anti A, anti B and anti D, were added and mixed with each blood sample and rocked gently for 60 sec to observe agglutination. There are differences in frequency distribution of the blood group (ABO) among the students. Hardy–Weinberg method was used to determine allelic frequencies and SPSS software was used for the data processing. Chi-square test was done to check whether the population was at Hardy-Weinberg genetic equilibrium based on ABO and Rh blood group phenotypes. In this study the most prevalent blood group was type O (40 %) followed by A (31.25 %), B (22.5 %), and AB (6.25 %). The most prevalent Rh-Positive blood groups among students were type O^+ (37.92 %) followed by A^+ (30 %), B^+(20 %), and AB^+ (5.42 %). Similarly the highest frequency in Rh-Negative blood groups among students was type O^- (6.25 %) followed by B^- (2.083%), A^- (1.25 %), and AB^-(0.83) blood group. Blood group AB and Rh-negative were least frequency among the students of Bora Secondary School. The distribution of allelic frequency was 0.2097 for I^A, 0.1561 for I^B and 0.6342 for I^O this occurred in the order of $I^O > I^A > I^B$. The allelic frequency of blood group O was the highest in the sample population and the genotypic frequencies were 0.4022 ($I^O I^O$), 0.04397 ($I^A I^A$), 0.2659 ($I^A I^O$), 0.02437 ($I^B I^B$), 0.1973($I^B I^O$) and 0.06546 ($I^A I^B$). Over all, the genotype of blood group $I^O I^O$ blood group was the highest.

Key words: Allelic frequency, genotypic frequency, ABO blood group and Rh (D) phenotypes

1. INTRODUCTION

Blood is a specialized connective tissue with complete and unchangeable identity. The ABO and Rh are recognized as the major clinically significant blood group antigens. The ABO system derives its importance from the fact that A and B are antigens and anti A and anti B occur naturally in the serum of persons lacking the corresponding antigens, these antibodies being capable of producing hemolysis in *vivo*. Rhesus blood group system was the fourth system to be discovered and yet it is the second most important blood group from the point of view of transfusion (Adeyemo *et al.,* 2006).

The discovery of the ABO blood group over 100 years ago caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood. As our understanding of the ABO blood group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now study one of the first human characteristics proven to be inherited. A person's ABO blood type was used by lawyers in paternity suits, by police in forensic science, and by anthropologists in the study of different populations (Tekade *et al.,* 2011).

Furthermore, the discovery of ABO and Rh blood group has contributed immensely to blood banking services and transfusion medicine in order to avoid morbidity and mortality in both adults and children. The distribution of the ABO and Rh blood group alleles vary worldwide and are not found in equal numbers. Among African-Americans the distribution of ABO blood group is, type O, 46%; type A, 27%; type B, 20%; and type AB, 7%. Among Caucasians in the United States, the distribution of type O is 47%; type A, 41%; type B, 9%; and type AB 3%; Among Western Europeans type O, 46%; type A, 42%; type B, 9%; and type AB, 3% (Pramanik, 2000).

The surface of our red blood cells contains different sugars and proteins called blood group antigens. Ability to form these substances is inherited. Humans have many blood group systems so that each individual has a unique spectrum of blood groups with exception of identical twins and triplets only. (Mayo, 2008)

The classification of blood into its groups is based on the presence or absence of inherited antigenic substance on the surface of red blood cells. Some of these antigens are also present on the surface of other types of cells and body secretions like saliva, sweat, tear, urine, semen, serum etc, which are used in forensic investigations. Several of these RBC surface antigens that stem from very closely linked genes collectively form blood group systems. Blood groups are genetically determined and exhibit polymorphism in different populations (Daniels, 2005).

A total of 30 human blood group systems are now recognized by the International Society of Blood Transfusion. In clinical practice ABO and Rh blood groups are the most important among 30 blood groups (Jaff, 2010).

The ABO blood group system is the most important blood group system in human blood transfusion. Found on platelets, epithelium, and cells other than erythrocytes, AB antigens (as with other serotypes) can also cause an adverse immune response to organ transplantation (Muramatsu *et al.*, 2014).

The associated anti-A and anti-B antibodies are usually IgM antibodies, which are produced in the first years of life by sensitization to environmental substances, such as food, bacteria, and viruses.ABO blood types are also present in some other animals, for example rodents and apes, such as chimpanzees, gibbons, and gorillas (Maton *et al.*,1993).

The blood plays more roles than one might expect, it is involved in respiration, nutrition, waste elimination, thermoregulation, immune defense, water and acid base balance, internal communication. Most adults have 4 to 6 L of blood containing

Erythrocytes (red blood cells), Leukocytes (white blood cells), and platelets. Erythrocytes have two principal functions: To pickup oxygen from the lungs and deliver it to tissues elsewhere and pickup carbon dioxide from other tissue and unload it in the lungs (Saladin, 2003).

The absence of specific antigens on erythrocytes or antibody in the serum of individual. Apart from their importance in blood transfusion practice, the ABO and Rh blood groups are useful in population genetic studies, researching population migration patterns as well as resolving certain medico-legal issues, particularly of disputed paternity cases. In modern medicine besides their importance in evolution, their relation to disease and environment is being increasingly important (Nwauche *et al.*, 2004)

The ABO and Rh blood groups are the most important blood group despite the long list of several other blood groups discovered so far. These blood groups are the most studied blood systems among human populations due to their clinical, genetic and anthropological importance (Bakare *et al.*, 2006).

The Rh blood group is determined by genes called D which has two alleles: D, d whatever other alleles a person may have, any one 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. Anti-D antibodies are not normally present in the blood. They form only in Rh negative individuals who are exposed to Rh positive 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 new born (HDN), or erythrblastosis fetlis (Saladin, 2003).

Blood grouping has improved with the advent of monoclonal antibodies and automation of test. Although different advanced techniques, such as micro plate method, PCR based typing, FMC based typing, mini sequencing analysis, florescent immune micro plate technique, sandwich methods, etc are available for ABO genotyping, the manual method has its significance not only in blood typing but also measuring its genotypic frequency by Hardy Weinberg law (Griffiths *et al*., 2008).

The ABO blood group system is governed by a single gene with the three alleles (I^A, I^B and I^O), of which I^A and I^B alleles are co- dominant but both of them are dominant over the recessive allele I^O. No previous study has been reported in the literature regarding the frequency of ABO alleles in the population of Bora district, like other countries in the world. Therefore the aim of this study is to investigate the frequency of ABO and Rh (D) alleles among students of Bora Secondary School, East Shoa Zone, Oromia Region Ethiopia.

General Objective

The general objective of this study is to determine the frequencies of ABO and Rh blood phenotypes, alleles and genotypes among students of Bora Secondary School.

Specific objectives

* To determine the frequency of the ABO and Rh (D) blood group phenotypes among students of Bora Secondary School.

* To determine the frequency of ABO and Rh (D) blood group alleles among Students of Bora Secondary School.

* To estimates the frequency of the ABO and Rh (D) blood group genotypes among students of Bora Secondary School.

2. LITURETURE RIEVEW

2.1. History of ABO Blood Grouping

The ABO and Rhesus (Rh) both remain the most important and famous blood group systems clinically. The ABO blood group system was first discovered in 1901 by, an Australian Scientist Karl Landsteiner. The Rh system was later described by both Landsteiner and Weiner in 1940 by their joint work. Both are equally important in clinical and forensic medicine. Since the discovery, attention of scientists has been greatly focused on these two blood group systems because of the fact that they are highly polymorphic and immunogenic especially in human (Avent, 2009).

Landsteiner named the first two blood group antigens A and antigen B, using the first two letters of alphabet while RBCs not reacting with anti A and anti B where called type C. Classification of blood group was based on his observation of the agglutination reaction between an antigen on erythrocytes and antibodies presents in serum of individuals directed against these antigens. Where no agglutination had occurred, either antigens or the antibodies were missing from the mixture. In 1902 Von Decastello and Sturi described RBCs reacting to both anti A and anti B but did not give these type a name, continued calling RBCs that did not react with anti-A and anti-B type C (Garrattey *et al.* ,2000).

In 1911, Von, Dungern and Hirszfeld 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. LudwikHirszfeld and Von Dugern described the heritability of ABO blood groups in 1910-1911.With Felix Bernstein demonstrating the correct blood group inheritance pattern of multiple alleles at one locus in 1924. Watkins and Morgan, in England discovered that the ABO epitopes were conferred by sugars, to be specific, N-acetylgalactosamine for the A-type and galactosyl for the B-type. After the literature claiming that the substances were all attached glycosphingolipid (Mollison, 1994).

2.2. Blood group systems

Blood types are classified in several ways. In the ABO system blood groups are classified as A, B, AB, and O. The Classification of blood as, Rh-positive and Rh-negative in Rh system is based on the presence or absence of inherited antigenic substance on the surface of the red blood cells. The antigens may be proteins, carbohydrates, glycoprotein and glaycolipids depending on the blood group system (Hasna *et al,* 2010).

A complete blood type would describe a full set of 30 substances on the surfaces of RBCs, and an individual`s blood types in one of many possible combinations of blood group antigens. Across the 30 blood group, over 600 different blood group antigens have been found, but many of these are very rare (ISBT, 2008).

Almost always, an individual has the same blood group for life, but very rarely individual`s blood type change by additional or suppression of an infection, malignancy, or autoimmune disease. Another more common in blood type change is bone marrow transplant. Bone marrow transplants are performed for many leukemia`s and lymphomas, among other disease. If a person with blood type-A bone marrow receives blood from patients of blood type-O, the patient`s blood type will eventually convert to the donor`s type (Masushita *et al*., 1983).

For instance early independent studies showed association of rectal, cervical, leukemia, pancreatic, breast, ovarian, gastric cancers among individuals with blood groups A or B more likely to have elevated risk of pancreatic cancer than individual belonging to blood group O (Greer, 2010).

2.3. Rh Blood Group System

The second major blood grouping system is the Rhesus (Rh) system. Like the ABO blood types, the Rh factor is an inherited blood protein, or antigen, on red blood cells. People who have it are "Rh positive"; those who don't are "Rh negative". The Rh system is more complex than the ABO system in that there are 35 different possibilities that can be inherited from each parent. These, however, are roughly grouped into positive and negative types. As shown on Table 1 being Rh positive is more common than being Rh negative: about 85% of people are Rh positive. Besides its role in blood transfusion, the Rh blood group system specifically, the D antigen is used to determine the risk of hemolytic disease of the new born (HDN) (Reid and Lomas, 2004).

A mother who is Rh-negative may develop antibodies to an Rh-positive baby. If a small amount of the baby's blood mixes with the mother's blood, which often happens in such situations, the mother's body may respond as if it were allergic to the baby. The mother's body may make antibodies to the Rh antigens in the baby's blood. This means the mother has become sensitized and her antibodies may cross the placenta and attack the baby's blood. Such an attack breaks down the fetus's red blood cells, creating anemia (Mais, 2009).

Table 1. Allelic frequency of Rh blood group studied in different population across the world

Population	Rh+	Rh-	References
	(%)	(%)	
Adymama(Nigeria)	0.9740	0.0260	Abdulazeez *etal*,(2008)
Lagos (Nigeria)	0.9400	0.0600	AdeyemoandSoboyeyi(2006)
Ogbomoso(Nigeria)	0.9670	0.0330	Bakar *et al*., (2006)
Benin (Nigeria)	0.9388	0.0603	EnosoleaseandBazuaye,(2008)
India	0.9445	0.0550	Khattak *et al.,* (2008)
Britain	0.8300	0.1700	Khattak *et al.,* (2008)
Germany	0.9500	0.0500	Akbase *et al.,* (2008)
USA	0.8500	0.1500	Khattak *et al.,* (2008)
Saudi Arabia	0.9300	0.0700	Khattak *et al.,* (2008)
Ethiopia	0.9464	0.0535	Seifu and Kifle,(1985)
Ibadan (Nigeria)	0.9500	0.0480	Omotad e *al*., (1999)
Ilorin (Nigeria)	0.0955	0.0450	Iyola,(2011)
Porthrcourt(Nigeria)	0.9677	0.0323	Jeremiah.,(2006)
Saudi Arabia	0.9300	0.0700	Khattak *et al.,* (2008)

Source: ISBT, 2008 (http://www.bloodbook.com/world-abo.html

2.3.1. Rh Antigens

Currently, 50 antigens have been described in the Rh group system; the D, C, c, E and e antigens are the most important. The others are much less frequently encountered or are rarely clinically significant. Rh or D is the most important antigen after A and B antigens. 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. Exposure to D+ cells usually occurs during pregnancy or transfusion (Reid and Lomas, 2004).

2.3.2 Rh Antibodies

The antibodies are in the blood plasma. Individuals have different types and combinations of these molecules. Rh antibodies are IgG antibodies which are acquired through exposure to Rh-positive blood (generally either during pregnancy or

transfusion of blood products). The D antigen is the most immunogenic of all the non-ABO antigens. Approximately 80% of individuals who are D-negative and exposed to a single D-positive unit will produce an anti-D antibody. The percentage of alloimmunization is significantly reduced in patients who are actively exsanguinations (blood loss to a degree, sufficient to cause death) (Cartron, 1999).

2.3.3. Allele and Genotype of ABO Blood Group

Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene (the ABO gene) with three of alleles: i, I^A, and I^B. The I^A allele gives type A, I^B gives type B, and i gives type O. As both I^A and I^B are dominant over i, only ii people have type O blood. Individuals with I^AI^A or I^Ai have type A blood, and individuals with I^BI^B or I^Bi have type B. I^AI^B people have both phenotypes, because A and B express a special dominance relationship; co dominance, which means that type A and B parents can have an AB child. A couple with type A and type B can also have a type O child if they are both heterozygous (I^Bi,I^Ai) The cis-AB phenotype has a single enzyme that creates both A and B antigens (Iyiola, 2011).

2.4. Distribution of ABO Blood Group

The ABO blood group system is the most important blood group system in human blood transfusion. The associated anti-A and anti-B antibodies are usually IgM antibodies, which are usually produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses. ABO blood types are also present in some other animals, in human, the majority of cell types investigated have A, B, or O antigen on their surfaces.This include some cells like platelet (Brian *et al*.,2000)

The ABO blood group distribution varies among the different people all over the world. For instance, Blood group B has its highest frequency in Northern India and neighboring Central Asia, and its incidence diminishes both towards the west and the east, falling to single digit percentages in Spain. It is believed to have been entirely absent from Native American and Australian Aborigines populations prior to the arrival of Europeans in those areas. Blood group A is associated with high frequencies in Europe, especially in Scandinavia and Central Europe, although its highest frequencies occur in some Australian Aborigines populations and the Blackfoot Indians of Montana (Dean, 2005).

2.5. Blood typing

As shown on Table 2 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. If clumping or clotting occurs in the test blood upon

exposure to the B antigen 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, the blood type is O (Avent, 2009).

Table 2. Agglutination reaction of ABO blood typing sera

Blood type	Reaction	
	A antibodies(anti-Aserum)	B antibodies(anti-Bserum)
A	Clumping	No clumping
B	No clumping	Clumping
AB	Clumping	Clumping
O	No clumping	No clumping

Source; Avent, 2009

2.6. Clinical significance of blood groups

2.6.1. Blood transfusion

Transfusion medicine is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of a blood bank to provide a transfusion service for blood and other blood products. Across the world, blood products must be prescribed by a medical doctor in a similar way as medicines. Much of the routine work of a blood bank involves testing blood from both donors 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 donor and recipient, a severe acute hemolytic reaction with hemolysis (RBC destruction), renal failure and shock is likely to occur, and death is a possibility. Antibodies can be highly active and

can attack 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 a transfusion reaction. Risks can be further reduced by 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 donor's red blood cells and checking if the mixture agglutinates, or forms clumps. If agglutination is not obvious by direct vision, blood bank technicians usually check for agglutination with a microscope. If agglutination occurs, that particular donor's blood cannot be transfused to that particular recipient (Bruce and May, 2002).

2.6.2. Blood group genotyping

As shown on Table 3 in addition to the current practice of serologic testing of blood types, the progress in molecular diagnostics allows the increasing use of blood group genotyping. In contrast to serologic tests reporting a direct blood type phenotype, genotyping allows the prediction of a phenotype based on the knowledge of the molecular basis of the currently known antigens. This allows a more detailed determination of the blood type and therefore a better match for transfusion, which can be crucial in particular for patients with needs for many transfusions to prevent allo-immunization (Anstee, 2009).

Table 3. Blood phenotype and genotype

Phenotype	Genotype
A	$I^A I^A$ or $I^A I^O$
B	$I^B I^B$ or $I^B I^O$
AB	$I^A I^B$
O	$I^O I^O$

Source; Anstee, 2009

2.6.3. Hemolytic Disease of the new born (HDN)

The hemolytic condition occurs when there is an incompatibility between the blood types of the mother and the fetus. There is also potential incompatibility if the mother is Rh negative and the father is positive. When any incompatibility is detected, the mother often receives an injection at 28 weeks gestation and at birth to avoid the development of antibodies toward the fetus. A pregnant woman can make IgG blood group antibodies if her fetus has a blood group antigen that she does not have. This can happen if some of the fetus' blood cells pass into the mother's blood circulation (e.g. a small fetomaternal hemorrhage at the time of childbirth or obstetric intervention), or sometimes after a therapeutic blood transfusion. This can cause Rh disease or other forms of hemolytic disease of the newborn (HDN) in the current pregnancy and/or subsequent pregnancies. 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 (Daniels, 2007).

One of the major advances of twentieth century medicine was to prevent this disease by stopping the formation of Anti-D antibodies by D negative mothers with an injectable medication called Rh (D) immune globulin. Antibodies associated with some blood groups can cause severe HDN, others can only cause mild HDN and others are not known to cause HDN (Letsky *et al*, 2000).

Due to its medical importance in relation to different diseases, pursuing a line of investigation on the ABO and Rh blood group systems has been of significance for years. It is well known that these blood group systems are of great importance in blood in transfusion and organ transplantation (Chandra , 2012)

2.6.4. Red blood cells compatibility

As indicated on Table 4 Blood group A individuals have the A antigen on the surface of their RBCs, and blood serum containing IgM antibodies against the B antigen.

Therefore, a group A individual can receive blood only from individuals of groups A or O (with A being preferable), and can donate blood to individuals with type A or AB (Simpkins and Williams, 1997).

Blood group B individuals have the B antigen on the surface of their RBCs, and blood serum containing IgM antibodies against the 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 and May, 2002).

Blood group AB individuals have both A and B antigens on the surface of their RBCs, and their blood plasma do not contain any antibodies against either A or B antigen. Therefore, an individual with type AB blood can receive blood from any group (with AB being preferable), but cannot donate blood to any group other than AB. They are known as universal recipients (Green walt, 1997).

Blood group O individuals do not have either A or B antigens on the surface of their RBCs, and their blood serum contains IgM anti-A 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, but can donate blood to individuals of any ABO blood group (i.e. A, B, O or AB). If a patient in a hospital situation needs a blood transfusion in an emergency, and if the time taken to process the recipient's blood would cause a detrimental delay, O negative blood can be issued (Griffiths *et al*, 2008).

Table 4 ABO blood phenotypes

Blood type	Antigens on erythrocytes	Antibodies in plasma	Received blood from groups	Give blood for 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

Source; Yazer *et al*, 2006

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Bora Woreda, East Shoa Zone, Oromia Regional State, Ethiopia. It is located at a distance of 110 km, from Addis Ababa and 38 km from Modjo and found in the Great Rift Valley, Modjo-Hawasa road. It is located at 80 8’ N, 380 57’E with an elevation of 1,611 meters above sea level.

The administrative center of Bora is Bote (AlemTena).The Population of this Woreda is 58,748, of whom 30,487are men and 28,261 are women; 11,403 or 19.41% of its population were urban dwellers. (Source; population and Housing Census of Ethiopia, 2007).

3.2. Study Population

This study was carried out at Bora Secondary School, Department of Natural Science, in Biology Laboratory at Bora Woreda, Oromia Zone, Ethiopia in 2015/2016. A total of 240 voluntary participant students were selected among 719 students enrolled for the academic year 2015/2016. The study was conducted on 240 sampled students of both sexes. These comprised of approximately 33.4% of the student population in the school. From the sample population, 134 (55.83%) were males and 106 (44.17%) were females which were selected for this study.

3.3. Blood Sample collection and Blood Group Determination

Blood typing was conducted during March 5-19/2016; a blood sample from each student was taken after they agreed to participate in the research and signed in the consent forms that assure their willingness. Blood was taken after wiping the middle finger with a piece of cotton saturated with 70% alcohol and piercing it with a sterile disposable lancet. A drop of blood was placed on three clean white glass slides on which a few drops of anti sera, anti A, anti B and anti D were applied. A drop of each of the anti sera anti A, anti B and anti D were added to an individual's blood samples and mixed using a glass rod and rocked gently for 60 sec to observe agglutination.

Blood group was determined on the basis of agglutination and recorded, as blood A^+, B^+, AB^+ O^+ or A^-, B^-, AB^-, O^-.The blood samples were collected and tested by qualified Laboratory technician using a standard clinical procedure with sterilized needles, slides and chemicals like anti-A, anti-B, anti-D (Bhasin and Chahal, 1996).

3.4. Observation of Blood Type

The following blood groups determined on the basis of agglutination reactions with antibodies.

A- Positive (A^+) blood type**;** A blood sample was determined as an A^+ blood type when agglutination reaction is observed with anti-A and anti-D as shown on Appendix Figure 1.

A- Negative (A^-) blood type**;** the blood sample was recognized as belonging to type A and Rh-negative if it agglutinates with anti-A but not with anti-D as indicated on Appendix Figure 2**.**

B- Positive (B^+) blood type**;** a blood sample was determined as B^+ blood type when agglutination reaction is observed with anti-B and anti-D as shown in Appendix Figure 3.

B- Negative (B^-) blood type**;** the sample was recognized as belonging to type B and Rh-negative if it agglutinates with anti-B with not with anti-D as shown in Appendix Figure 4.

AB- Positive (AB^+) blood type**;** A blood sample was determined as AB^+ blood type is determined when agglutination reaction is observed with anti-A, anti-B and anti-D as shown in appendix Figure 5.

AB- Negative (AB^-) blood type; the blood sample was recognized as belonging to AB and Rh-negative if it agglutinates with anti-A, anti-B but not with anti-D as shown in Appendix Figure 6.

O- Positive (O^+) blood type**;** a blood sample was determined as an O^+ blood when agglutination reaction observed with anti-D but not in anti-A and anti-B as shown in Appendix Figure7.

O - Negative (O^-) blood type**;** The blood sample was recognized as belonging to type O and Rh-negative if it does not agglutinate with all in anti-A, anti-B and anti-D as shown in Appendix Figure 8.

3.5. Statistical Analysis

The data were entered to Microsoft excel and then statistical package SPSS version 16.0 was used for data analysis. Allele frequencies were calculated under the assumption of Hardy–Weinberg equilibrium. For this study, the three ABO blood

group alleles are represented as I^A, I^B and I^O with frequencies of p, q and r, respectively (Griffiths *et al*., 2008).

Preliminary estimates for the frequency of the three alleles were computed as:

$P=1-\sqrt{B+O}$

$q=1-\sqrt{A+O}$

$r=\sqrt{O}$

Where p= frequency of the I^A allele

q=frequency of the I^B allele and

r=frequency of the I^O allele

and A, B and O are observed frequencies of the blood groups A, B and O respectively. A correction factor d was calculated as

$d=1-p-q-r$

The final adjusted frequencies of the three alleles

$p' = p (1+ d/2)$

$q'= q (1+ d/2)$

$r'= r (1+d/2) (1+d/2)$

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).

Where: $P^2 = I^A I^A$ - homozygous genotype for blood type A.

$2pq=2I^AI^B$-heterozygous genotype for blood type AB.

$q^2 = I^B I^B$ - homozygous genotype for blood type B.

$2pr=2I^A I^O$- heterozygous genotype for blood type A.

$2qr=2I^B I^O$ - heterozygous genotype for blood type B.

$r^2 = I^O I^O$ - homozygous genotype for blood type O.

The chi square test was done to test whether the population was at Hardy Weinberg genetic equilibrium based on the ABO and Rh blood group phenotype.

The χ^2 test first calculates a χ^2 statistic using the formula:

$$\chi^2 = \sum_{i=1}^{r}\sum_{j=1}^{c}\frac{\left(A_{ij} - E_{ij}\right)^2}{E_{ij}}$$

Or $= \underline{(O-E)^2}$

E

Where: Aij = actual frequency in the i-th row, j-th column

Eij = expected frequency in the i-th row, j-th column

r = number or rows

c = number of columns

4 RESULTS AND DISCUSSION

4.1. Distribution of Blood Group Phenotypes among Students of Bora Secondary School

4.1.1 Frequency of ABO blood group phenotypes among the Students of Bora Secondary School

Table 5. Frequency of ABO blood group phenotypes among the Students

Sex	ABO System				Total
	A	B	AB	O	
Male	35(26.12%)	32(23.88%)	9(6.72%)	58(43.28%)	134
Female	40(37.74%)	22(20.75%)	6(5.66%)	38(35.85%)	106
Total	75(31.25%)	54(22.5%)	15(6.25%)	96(40%)	240

There are differences in frequency distribution of the ABO blood group phenotypes as shown in Table 6. The frequency of ABO blood types among the students of Bora Secondary School were 75(31.25%), 54 (22.5%), 15 (6.25%) and 96 (40%) for blood groups A, B, AB and O, respectively. Over all the most prevalent blood group was type O and the least frequent was AB. The distribution of blood group A was higher among the female Students than the males, occurring with frequency of 37.74% and 26.12% respectively in males and females. The differences between male and female were due to having difference between sexes.

Normally, the frequency of ABO blood group varies from one population to another. In many other studies, blood group O has been found to be the most common blood group.

Among the Caucasians in the United States, the distribution is group O, 47%, group A, 41%, group B, 9% and group AB, 3% Among Western Europeans 42% are group A, 9% group B,3% group AB and the remaining 46% group O (Adeyemo and Soboyejo,2006).

As the previous study reports, the distribution of ABO blood group in Ethiopian were O,40%; A, 31%; B, 23%; and AB; 6% (Misganaw, 2004).

This is in agreement with this study. However the present finding is deviate from the results obtained by Khan and his colleagues on the phenotype frequencies of blood groups from Bannu region in Pakistan where ABO blood group frequency occurred in the order of B>A>O>AB (Khan *et al.*,2009).

4.1.2. Frequency of Rh (D) blood Group phenotype

The distribution of Rh negative was very low among the students of the School Rh (D) blood group system, the distribution of Rh phenotypes were, Rh-positive 225(93.75%) and Rh-negative 15(6.25%). In other studies Rh negative blood group is documented as 5.5% in south India, 5% in Nairobi Kenya, 4.5% in Nigeria, 7.5% in Lahore, 7.7% in Rawalpindi studies (Khan, et *al*., 2009).

Table 6. Phenotypic frequency of Rhesus factor blood group among students

SEX	Rhesus system		Total
	Rh+%	Rh-%	
Male	127(94.78%)	7(5.22%)	134
Female	98(92.45%)	8(7.55%)	106
Total	225(93.75%)	15(6.25%)	240

There are differences in frequency distribution of the Rh blood group phenotypes. As indicated in Table 7 the frequency of Rh blood group phenotypes among the students of Bora Secondary School Overall frequency for Rh- positive was 225 (93.75%), had

higher frequency than Rh-negative 15(6.25%). Female had higher frequency for Rh-negative 8(7.55%) than male7 (5.22%). The differences between male and female were due to having difference between sexes.

4.2. Frequency of the blood groups alleles among the students of Bora Secondary School

4.2.1. Frequency of ABO blood group alleles among the students of Bora Secondary School

Table 7. ABO blood group allelic frequencies among students

ABO Phenotype	Observed number	Phenotypic Frequency	Allele	Allele frequency
A	75	0.3125	I^A	0.2095
B	54	0.225	I^B	0.1559
AB	15	0.0625	-	-
O	96	0.4	I^O	0.6325

After computing the correction factor d as d=1-p-q-r, the frequencies of the blood group alleles are adjusted as follows to obtain the final frequencies p', q' and r'.

$p' = p (1+ d/2)$

$p' = 0.2095(1+0.0021/2) = 0.2097$

$q' = q (1+ d/2)$

$q' = 0.1559 (1+0.00105) = 0.1561$

$r' = r (1+d/2) (1+d/2)$

$r' = (0.6325+0.00105) (1+0.00105) = 0.6342$

Table 8 shows the distributions of the ABO blood groups and allelic frequencies among students Bora Secondary School. The estimated allele frequencies were 0.2095 for A, 0.1559 for B and 0.6325 for O. This occurred in the order of O>A>B. The

allele frequency of blood group O was the highest in the sampled population (Chakraborty, 2010).

4.2.2. Frequency of Rh blood Group alleles among the Students Bora Secondary School

Table 8. Frequency of Rh blood Group alleles among the students

Phenotypes	Observed number	Allelic frequencies
Rh(D) positive	225	0.9375
Rh(D) negative	15	0.0625
Total	240	1.00

As indicated in table 9, the Rh (D) positive allelic frequency was 0.9375 and the Rh (D) negative was 0.0625 recorded. The frequencies of Rh (D) positive were greater than in Bora Secondary School students.

4.3. Frequency of ABO and Rh (D) blood group genotypes among Bora Secondary School

4.3.1. Frequency of ABO blood group genotype among Students of Bora Secondary School

An important application of the Hardy–Weinberg principle is estimating the heterozygous frequencies in a population. To calculate the frequency of individuals who have heterozygous recessive traits, we usually begin by counting the number of homozygous recessive individuals. These homozygous individuals can be distinguished from the rest of the population by clinical symptoms that indicate the defects.

For this study, the frequencies of the ABO blood group genotype and alleles were calculated using the extension of the Hardy–Weinberg principle (Griffith *et al*, 2008).

$(p+ q + r)^2 = p^2$ (AA) +2pq (AB) $+q^2$ (BB) +2pr (AO) +2qr (BO) + r^2 (OO).

In this system, the alleles A and B are co-dominant and both are dominant to O. This system has six possible genotypic combinations but only four phenotypic blood groups.

Table 9. Allelic and genotypic frequencies of ABO blood group alleles among Students

Blood group	Allele	Frequency	Genotype	Frequency	Pheno type	Frequency%
ABO	I^O	0.6342	$I^O I^O$	0.4022	O	40.2
	I^A	0.2097	$I^A I^A$	0.04397	A	31
			$I^A I^O$	0.2659		
	I^B	0.1561	$I^B I^B$	0.02437	B	22.2
			$I^B I^O$	0.1973		
			$I^A I^B$	0.06546	AB	6.6

As shown in Table 10, most of the A and B blood types are heterozygous in the sample population. Heterozygous genotype $I^A I^O$ is more frequent than heterozygous $I^B I^O$, the results are in agreement with the finding of (Bakare *et al., 2006),* that the predominance of O allele may also be 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 $I^A I^A$ was more frequent than homozygous blood group $I^B I^B$. The genotype frequencies were 0.4022 ($I^O I^O$), 0.04397 ($I^A I^A$), O.2659 ($I^A I^O$), 0.02437 ($I^B I^B$), 0.1973 ($I^B I^O$) and 0.06546 ($I^A I^B$). The genotype blood group $I^O I^O$ was the highest in among the sampled population.

4.3.2. Frequency of Rh (D) blood group genotype among students. Bora Secondary School

Table 10. Frequency of Rh (D) blood group genotype among students.

Blood group	Allele	frequency	Genotype	Frequency	Phenotype
Rhesus	D	0.75	DD	0.5625	Rh(D) +ve
			Dd	0.375	Rh(D) +ve
	D	0.25	dd	0.0625	Rh(D)-ve

As indicated in Table 11, with regards to Rhesus D antigen, the phenotypic proportions to be 225(0.75) for Rh positive which consistof genotypic frequency of DD (0.5625), Dd(0.375) and phenotypic proportions to be 15(0.25) for Rh negative which consisted genotypic frequency of dd(0.0625).This study is in agrmeent with Bakare(**). From previous studies among Nigerian population where the Rh positive were found to be higher in the population than Rh (D) negative (Bakare *et al.*,2006).

4.4. ABO blood group phenotype based on Rh (D) factor

In this study, it was observed that blood group O positive is the highest with a frequency of 91(0.379) which is followed by group A positive with the frequency of 72(0.3), blood groups B positive is 49(0.204) and AB positive 13 (0.052) This results showed a total frequency of Rh positive distribution as 225(0. 9375) and Rh negative *d*istribution to be 15(0.0625). It can be seen that blood group AB has the least percentage; which is most of the time very rare and also the case in other previous studies Rhesus blood group distribution also varies with in any group of human population to others (Akbas, *et al*., 2003).

Table 11. ABO blood group phenotype based Rh (D) factor among Students of Bora Secondary School

	Blood types				**Total**
	A	B	AB	O	
Positive	72(0.3)	49(0.204)	13(0.052)	91(0.379)	225(0.93)
Negative	3(0.013)	5(0.021)	2(0.0083)	5(0.02)	15(0.063)

As indicated in table 12, the most prevalent Rh-positive blood groups among Students was type O^+ (0.3792) followed by A^+ (0. 3), B^+ (0.2042) and AB^+ (0.0542) and O^- (0.0208) and B^- have the same frequency followed by A^- (0.0125) and AB^- (0.0083) . This study is in agreement with (patel *et al*., 2012) had reported that looking at the rhesus grouping, on an average (0.9375) were Rh positive and remaining (0.0625) were Rh negative.

4.5. Chi Square Test for the ABO Blood Group Phenotypes in Bora Secondary School Students

Table 13. Chi square test for the ABO blood group phenotypes

Blood group	observed	expected	Deviation(d) (O-E)	d^2	d^2/E
A	75	74.3899	0.6102	0.3723	0.0050
B	54	53.3675	0.6325	0.4001	0.0075
AB	15	15.71241	0.7124	0.5075	0.3230
O	96	96.5303	0.5303	0.2812	0.0029
				P value= 0.9	x^2=0.3384

.

As shown in table 13, the ABO blood group phenotypes distribution was calculated by chi- square test at p value<0.05, 95% confidence level .In this study the chi-square test in ABO blood group was 0.3384 which has the p value is between 0.9 with 1 degree of freedom .there is no significance difference in the distribution of ABO blood group in Bora Secondary School students.

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

The research was conducted in Bora Secondary School, Bora Woreda, East Shoa Zone, Oromia region. The researcher was aimed to providing information on the frequency pattern of phenotypes, genotypes and the allelic frequency of ABO and Rh blood groups among students of Bora Secondary School.

A total of 240 Blood samples were collected from a total of 240 voluntary participant students. Blood sample was determined by open slide test method between March 5-19/2016. A drop of each anti sera; anti-A, anti-B and anti-D was added to an individual's blood samples and mixed using a glass rod and rocked gently for 60 sec to observe agglutination.

Hardy–Weinberg method was used to determine allelic frequencies and SPSS software was used for the data processing. Whereas chi-square test were used whether the population at Hardy-Weinberg Genetic equilibrium based on ABO and Rh Blood Group Phenotypes.

The most prevalent blood group frequency was type O (40 %) and also in Rh-Positive blood group frequency was type O+ (37.92 %), while blood group AB and Rh-Negative blood group are least frequent among the students. The allele frequency I^O and genotype frequency I^O I^O of blood group O was the highest in the sampled population.

Knowledge of the distribution of ABO, Rh blood groups in any population is useful in health care planning, medical diagnosis of anemia, allocation of resources and targeting the population that need counseling. If such information is well managed it can make a difference in the quality of decisions that

individuals will make especially as it concerns marriage, blood transfusion , genetic counseling and in general physiological wellbeing of individuals in a population. And also very important during emergency and accidental health disorder especially, for donating blood. Additionally this information would be useful to geneticist and to clinicians in planning of blood transfusion programmes since they play integral part in one's genetic profile.

5.2. Conclusion

In this study the most prevalent blood group was type O (40 %) followed by A (31.25 %), B (22.5 %), and AB (6.25 %). The most prevalent Rh-Positive blood groups among students were type O^+ (37.92 %) followed by A^+ (30 %), B^+(20 %), and AB^+ (5.42 %) as well as the highest frequency in Rh-Negative blood groups among students was type O^- (6.25 %) followed by B^- (2.083%), A^- (1.25 %), and AB^-(0.83) blood group. Blood group AB and Rh-negative were least frequency among the students of Bora Secondary School.

The distribution of allelic frequency was 0.2097 for I^A, 0.1561 for I^B and 0.6342 for I^O this occurred in the order of $I^O > I^A > I^B$. The allele frequency of blood group O was the highest in the sample population.

The genotype frequencies were $I^O I^O$ (0.4022), $I^A I^A$ (0.04397), I^A IO (O.2659), $I^B I^B$ (0.02437), $I^B I^O$ (0.1973) and $I^A I^B$ (0.06546). Over all, the genotype blood group $I^O I^O$ blood group was the highest.

The data generated would be helpful as a base for researchers who are interested to conduct similar type of study in Bora Woreda, East Shoa Zone, and Oromia Region.

5.3. Recommendations

- Based on the finding of this study the following points are recommended to investigate more on frequencies of ABO and Rh (D) blood group alleles in Bora Woreda.

- It is necessary to raise awareness among students the importance of blood typing and determination.

- Results revealed that due to limitation of resources the sample size used to conduct this study was small and may not represent the number of population in Bora Woreda .Therefore it is advisable to use large sample size to obtain more accurate data regarding the pattern of distribution on these blood groups.

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APPENDIX FIGURES

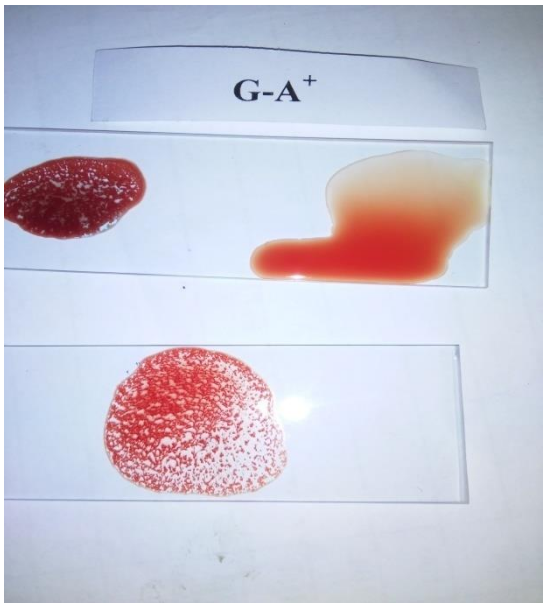

Figure 1 A Positive blood group sample

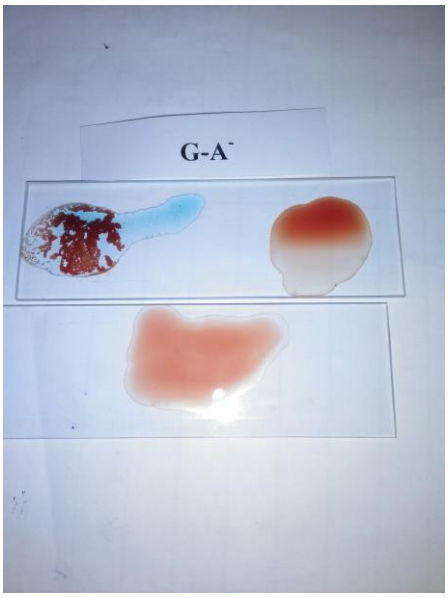

Figure 2 A negative blood group samples

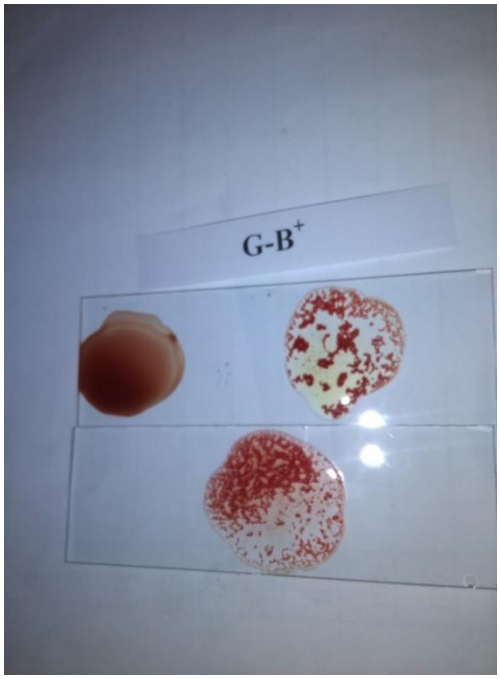

Figure 3 B positive blood group samples

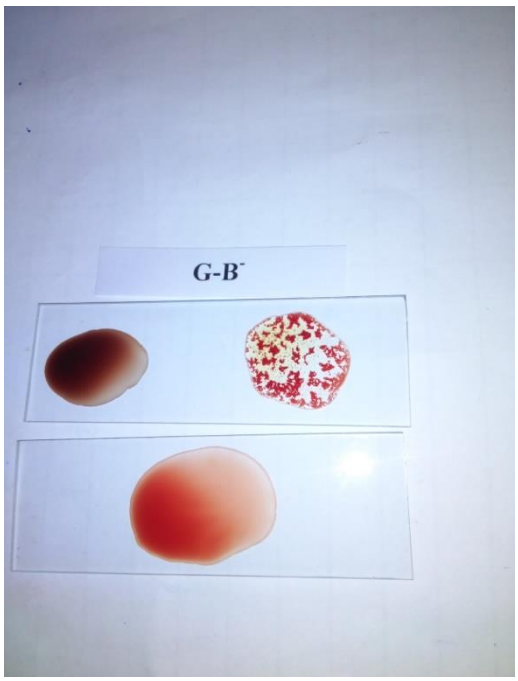

Figure 4 B negative blood group samples

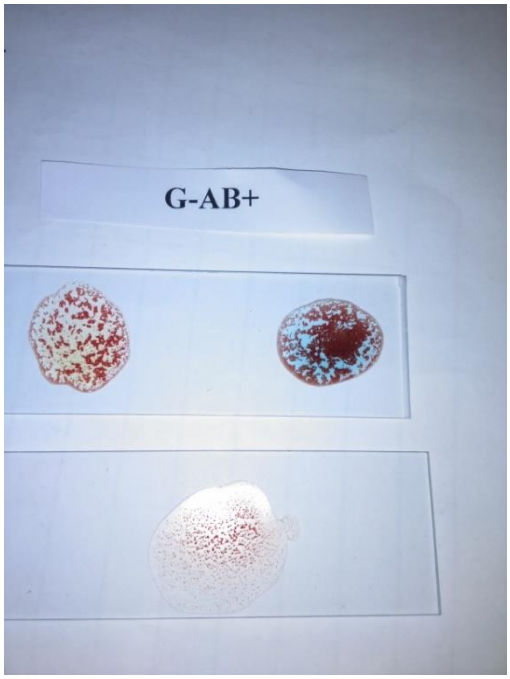

Figure 5 AB positive blood group samples

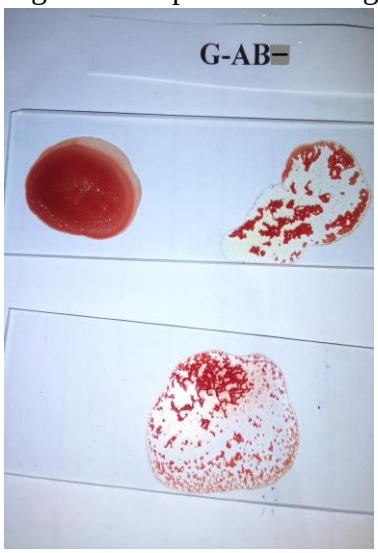

Figure 6 AB negative blood group samples

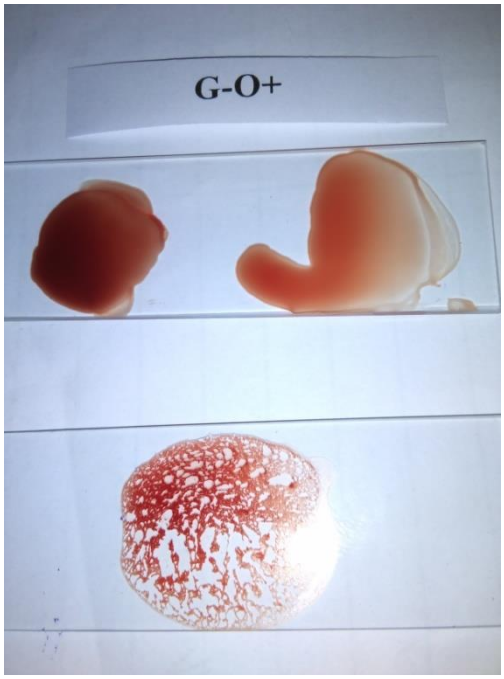

Figure 7 O positive blood group samples

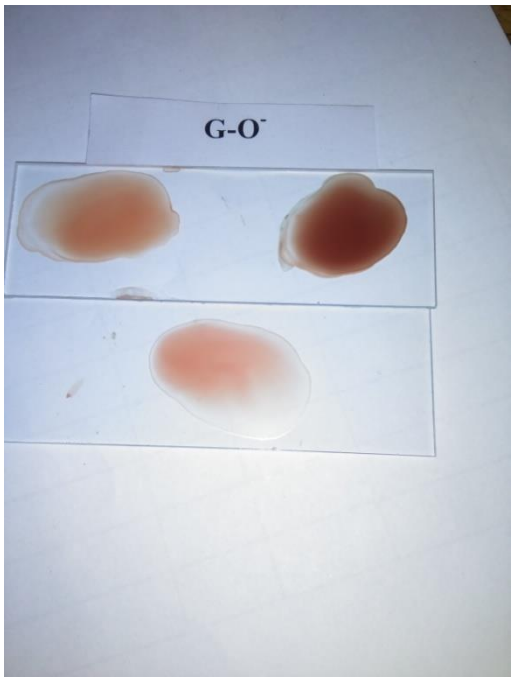

Figure 8 O negative blood group samples

Figure 9. During blood sample collection from volunteer students

Figure 10. Anti-A Anti-B Anti-D

Figure11.Determination of blood type results after added anti sera on Slides

Consent form

My name that listed below have been informed and understand the purpose of this research project is to find out the frequency of ABO and blood group alleles among students of Bora Secondary School, Bora Woreda ,East Shoa Zone. I have also been informed that the information that is obtained from me will be treated with self-assurance. Furthermore, I have been told that I can refuse to participate in the study. Hence with this understanding, I hereby agree to participate in this particular research voluntarily.

Name of student:_____

Age_____

Signature_____

Date_____

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