PREVALENCE OF UNEXPECTED RED CELL ANTIBODIES IN HEALTHY DONOR POPULATION IN A TERTIARY CARE CENTER IN SOUTH KERALA

Dissertation submitted to

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM

In the partial fulfilment of the requirement for the degree for

M.D. in Transfusion Medicine

By

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Under the Guidance of

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2017 – 2019

DECLARATION BY THE CANDIDATE

*I hereby declare that this dissertation titled "**Prevalence of Unexpected Red Cell Antibodies in Healthy Donor Population in a Tertiary Care Center in South Kerala"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. Debasish Gupta**, Professor and Head, Department of Transfusion Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum.*

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Place: ***Trivandrum***

Date: ***30th June 2019***

CERTIFICATE BY THE GUIDE

*This is to certify that the dissertation titled "**Prevalence of Unexpected Red Cell Antibodies in Healthy Donor Population in a Tertiary Care Center in South Kerala",** is a bonafide research work done by Dr. Gayathri A M in partial fulfilment of the requirement of the degree of MD Transfusion Medicine under my guidance and supervision.*

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ACKNOWLEDGEMENT

Thanks to Almighty for giving me strength and ability to understand, learn and completion of my ventures. With boundless love and appreciation, I would like to extend my heartfelt gratitude to the people who helped me to bring this study into reality.

I consider myself fortunate indeed to have had the opportunity to pursue my post-graduation and completion of MD thesis under the guidance of one of the most prestigious personalities in the field of Indian Transfusion Medicine, **Prof. Debasish Gupta**, Professor and Head, Department of Transfusion Medicine, SCTIMST, Trivandrum. His dedication and keen interest above all his overwhelming attitude to help his students had been solely and mainly responsible for completing my work. His timely advice, meticulous scrutiny, scholarly advice and scientific approach have helped me to a very great extent to accomplish this task.

I owe a deep sense of gratitude to **Dr. P V Sulochana** and **Dr. S Sathyabhama**, Department of Transfusion Medicine, SCTIMST, Trivandrum for their keen interest on me at every stage of my research. Their prompt inspirations, timely suggestions with kindness, enthusiasm and dynamism have enabled me to complete my thesis. I am deeply indebted to **Dr. Jissa V T**, Scientist C, Achutha Menon Centre for Health Science Studies, Trivandrum, who helped me with the statistical analysis of the data.

I would like to express my gratitude to **Dr. Raj Bharath R** and **Dr. Amita R**, Associate and Assistant Professors, Department of Transfusion Medicine, SCTIMST, for their valuable suggestions and tremendous support.

I extend my respect to my seniors' **Dr. Revathy Nair** and **Dr. Vinu Rajendran** for their valuable advice and unconditional support as well as my affection to my juniors' **Dr. Anila Mani** and **Dr. Sreethu Chand** for their constant help and support for completing my thesis work. I am deeply indebted towards **Mrs. Sheela Devi K S** and **Mrs. Sindhu P N**, Scientific Officers, Department of Transfusion Medicine, SCTIMST and to all other Technical Assistants, Unit helpers and Diploma students of the Department of Transfusion Medicine for their unconditional support for completion of my thesis.

My family is my circle of strength, faith and ultimate heaven guarded by my mother **Smt. Ajithakumari V M**, my father **Sri. Mohanan Nair C**, my sister **Ms. Gopika A M**, my grandmother **Smt. Madhavi Amma**, my better half **Adv. Abhilash R** and my little prince **Advaith Krishnan**. Without their support I would not have achieved my academic well-being.

Last but not the least, I express my heartfelt gratitude to the study participants who voluntarily agreed to be part of my study and without them this thesis would have been infeasible.

Dr. Gayathri A M

CONTENTS

ABBREVIATIONS

AABB	American Association of Blood Banks
AHG	Anti-Human Globulin
AHTR	Acute Hemolytic Transfusion Reaction
AIHA	Autoimmune Hemolytic Anemia
ALI	Acute Lung Injury
APAb	Anti-Phospholipid Antibody
BCA	Benign Cold Agglutinins
C3d	Complement Component 3
CA	Cold Agglutinins
CAT	Column Agglutination Technology
CTT	Conventional Tube Technique
DAT	Direct Antiglobulin Test
DGHS	Directorate General of Health Services
DHTR	Delayed Hemolytic Transfusion Reaction
DNA	Deoxy-ribo nucleic acid
DTT	Dithiothreitol
HDFN	Hemolytic Disease of Fetus and Newborn
HLA	Human Leucocyte Antigen

IAT	Indirect Antiglobulin Test
Ig	Immunoglobulin
IRL	Immunohematology Reference Laboratory
ISBT	International Society of Blood Transfusion
IVIG	Intravenous Immunoglobulin
MHLBI	National Heart, Lung, and Blood Institute
NACO	National AIDS control Organisation
PBS	Phosphate-buffered saline
PMN	Poly morpho nuclear cells
PRBC	Packed red blood cells
RBC	Red blood cell
REDS III	Recipient Epidemiology and Donor Evaluation Study-III
RNA	Ribo-nucleic acid
rpm	Rotations per minute
SC	Screening cells
SPRCA	Solid Phase Red Cell Adherence
TRALI	Transfusion Related Acute Lung Injury
TTI	Transfusion Transmitted Infections

INTRODUCTION

Naturally occurring anti-A and anti-B are the only red cell antibodies that are commonly found in human serum or plasma. All other antibodies are called “unexpected red cell antibodies [1]. There are two types of unexpected red cell antibodies: alloantibodies and auto-antibodies. Alloimmunization occurs because of red cells antigenic differences between donor and recipient in previous transfusions or between mother and fetus. Auto-antibodies are those produced against one’s own antigens. Immune humoral response in the presence of autoantibodies against intracellular antigens characteristically occurs in a majority of connective tissue diseases namely systemic lupus erythematous, systemic sclerosis, Sjögren syndrome, mixed connective tissue disease, polymyositis, and dermatomyositis [2]. Presence of these antibodies, alone or in combination, makes difficulties with compatibility testing, thereby delaying in issue of a compatible blood unit or may reduce post transfusion RBC life span [3].

The compatibility test comprises ABO/Rh determination, antibody screen and cross-match. Type & cross-match technique/Immediate spin cross match is routinely practised now which only detects ABO incompatibility between donor RBCs and recipient serum/plasma. Type & screen method is performed only when the recipient has unexpected alloantibodies to detect additional RBC incompatibility [4]. Because of the presence of auto-antibodies, all crossmatches become incompatible. Studies conducted based on these unexpected antibodies have largely concentrated on multiply transfused patient populations or antenatal women. Alloimmunization in these groups has a reported incidence up to 60 percent, with an up to fourfold increased risk of multiple antibodies compared to the risk of single antibodies [5]. However, such studies related to healthy donor population are not done extensively. The incidence of RBC alloimmunization depends on the demography and characteristics of the

population being studied. The specificity, Ig class, thermal range and concentration of the antibody can predict its clinical significance as well as patient's individual immune response is also significant factors. The balance between sensitivity and specificity can be influenced by the methods and technologies selected. It is not possible to detect all potentially clinically significant antibodies, or to avoid detecting all clinically insignificant antibodies [6].

The Direct Antiglobulin test (DAT) is a simple test used to determine if red cells have been coated in vivo with immunoglobulin (Ig), complement or both. It is used primarily for the investigation of hemolytic transfusion reactions, haemolytic disease of the fetus and newborn (HDFN), autoimmune hemolytic anemia (AIHA), and drug-induced immune hemolysis. An indirect antiglobulin test (IAT) is used to detect and identify unexpected antibodies in the serum of blood donors, prospective transfusion recipients, and prenatal patients [7]. IAT detect in vitro antibody-antigen reactions and detect very low concentrations of antibodies present in an individual's plasma/serum.

When unexpected antibodies are present, as indicated by positive screening tests, they must be identified. At a minimum, this involves testing the patient's serum against a panel of fully phenotyped reagent red cell samples as well as the patient's own cells [6]. A recent study suggests that a positive DAT result in a healthy blood donor may be a marker of risk of future development of malignancy [8]. All these point towards the need of Type & Screening system to be followed routinely in transfusion practices rather than Type & Matching system. Antibody screening is mandatory as laid down by Drug and Cosmetic Act 1940 and Directorate General of Health Services (DGHS) guidelines. Hence through this thesis work, we are

implementing routine antibody screening along with DAT testing in every donated units in our Institute.

Our donor pool consists of 100% voluntary regular donors who are considered to be absolutely safe and almost free of any infections and highly motivated. This study also helps to find the prevalence of irregular antibodies in such healthy donors there by aiding best transfusion practices to be followed in the institution since there is scarce data available on prevalence and type of irregular antibodies in Indian donor population.

AIMS AND OBJECTIVES

To study the prevalence of Unexpected Red Cell Antibodies in Healthy Donor Population in a Tertiary Care Centre in South Kerala with the following objectives:

1. To establish unexpected red cell antibody screening in voluntary blood donors as a routine mandatory practice in the Institute
2. To ensure blood components issued to patients are safe and less likely to cause any red cell antibody mediated transfusion reactions in the recipient
3. To add information about the prevalence of unexpected red cell antibodies from south India to the national database

REVIEW OF LITERATURE

Just as genetic information determines the colour of one's eyes, it also plays an important role in determining the blood groups expressed. The term blood group is usually restricted to blood cell surface antigens, and generally to red blood cell (RBC) surface antigens. Blood groups are inherited in *Mendelian* fashion. Each parent contributes half of the inheritance and genetic information is carried on double strands of deoxyribonucleic acid (DNA) known as *chromosomes*. Human cells have 23 pairs of chromosomes in the cell nucleus and out of these, 22 pairs are autosomes and 1 pair of sex chromosomes. The units that code for various expressions of inherited genetic information are known as *genes* which are found in specific places along the chromosomes termed *loci*. For each locus, there may be several different forms of a gene, which are known as *alleles.* When both the inherited alleles are identical, the person is *homozygous* and if different, the person is *heterozygous*. Sometimes, homozygous inheritance produces a stronger expression of the gene than would be seen in a heterozygous individual. This stronger expression is known as *dosage* and is important because some antibodies react more strongly with RBCs homozygous for a particular blood group inheritance than with those with heterozygous inheritance.

The concept of population genetics of blood group antigens plays an important role in blood banking. The term *genotype* is the set of genes in our DNA which is responsible for a particular trait (detectable products) and *phenotype* is the physical expression, or characteristics, of that trait. Based on mode of inheritance, whether dominant or recessive in autosomes or sex chromosomes decide the expression of antigens on the cell surface of the individual. Phenotype frequencies in a population are determined by randomly testing RBCs from a large number of individuals of that particular population. The percentage of those positive (or negative) for a particular

trait is then calculated and indicates the frequency of a particular phenotype (percentage or decimal). Once the individual frequencies of traits are known, it is possible to calculate the frequency of multiple traits by multiplying the frequencies of each trait. When two alleles are involved, it is possible to use the Hardy–Weinberg equation to determine the frequency of each allele. This equation states the following: $p2 + 2pq + q2 = 1$. The frequency of an allele in the population is represented by the sum of the frequency of the allele in the homozygous state plus the frequency of the allele in the heterozygous state (9).

$$\text{Frequency of } p = p2 + 2pq$$

An exception to Mendelian law known as linkage, if two genes are linked, they do not assort independently. Linked genes are located close to each other on the same chromosome, and there is a 50% or greater chance that they will remain together and be passed to the same gamete. Several blood group genes have been found to be linked to other genes. The linkage of the locus for the Lutheran blood group trait to the locus for the secretor trait was the first known example of autosomal linkage in humans, reported by Mohr in 1951. Because the genes are not inherited independently of each other, they are not in equilibrium and hence known as linkage disequilibrium.

An antigen is a substance that is capable of reacting with the product of an immune response. Antigen combines with antibody (i.e., "antigen–antibody reaction". Introduction of nonself immunogens present on human RBCs, white blood cells, and platelets may elicit antibody production. On a biochemical level, an immunogen is a substance with a molecular weight of 10,000 D or more. Substances with a molecular weight of less than 5,000 D seldom cause antibody formation. If coupled with larger carrier

molecules, however, these substances, known as *haptens*, can become immunogenic (able to stimulate an immune response). Once an immune response has been initiated by the hapten–carrier complex, the hapten alone can react with the product of the immune response (i.e., antibody). Complex biochemical compounds, especially those containing proteins or polypeptide– carbohydrate combinations, are highly immunogenic. The more diverse the molecule is, the more immunogenic it becomes. As previously indicated, the structure must be recognized by the cells of the recipient's immune system to be non-self.

Biochemical analysis of blood group antigens has shown that they fall into two main types: (i) protein determinants, which represent the primary products of blood group genes; and (ii) carbohydrate determinants on glycoproteins and glycolipids, in which the products of the genes controlling antigen expression are glycosyltransferase enzymes (9). Some antigens are defined by the amino acid sequence of a glycoprotein, but are dependent on the presence of carbohydrate for their recognition serologically. In recent years, molecular genetical techniques have been introduced into the study of human blood groups and now most of the genes governing blood group systems have been cloned and sequenced. Many serological complexities of blood groups are now explained at the gene level by a variety of mechanisms, including point mutation, unequal crossing-over, gene conversion, and alternative ribonucleic acid (RNA) splicing. Currently, there are 346 recognized blood group antigens identified, out of which 308 are clustered within 36 blood groups systems. These blood group antigens have the ability to mount immune challenge and result in antibody production.

INCIDENCES OF IRREGULAR ANTIBODIES

The detection of antibodies directed against red cell antigens is critical in pretransfusion compatibility testing methods such as Conventional Tube Technique (CTT), Column Agglutination Technique (CAT) and Solid Phase Red Cell Adherence (SPRCA) [10]. It is one of the principle tools for investigating potential hemolytic transfusion reactions and immune hemolytic anemias. In addition, it aids in detecting and monitoring patients who are at risk of delivering infants with HDFN. The focus of antibody detection methods is on "irregular" or "unexpected" antibodies, as opposed to the "expected" antibodies of the ABO system. There are many different and important characteristics of blood group antibodies, such as whether they are polyclonal or monoclonal, naturally occurring or immune, and alloantibodies or autoantibodies [9]. An antigen usually consists of numerous epitopes, and it is the epitopes and not the entire antigen that a B cell is stimulated to produce antibody against. Hence different epitopes of same antigen produce individual antibodies resulting in polyclonal antibody production. Monoclonal antibodies are prepared in controlled environment through hybridoma technology. The unexpected antibodies of primary importance are the immune alloantibodies, which are produced in response to RBC stimulation through transfusion, transplantation, or pregnancy. Other unexpected antibodies may be "naturally occurring" (i.e., produced without RBC stimulation). Naturally occurring antibodies may form as a result of exposure to environmental sources, such as pollen, fungus, and bacteria, which have structures similar to some RBC antigens. Antibodies produced in one individual and then transmitted to another via plasma-containing blood components or derivatives such as intravenous immunoglobulin (IVIG) are known as passively acquired antibodies, a third category of unexpected antibody. The presence of naturally occurring and

passive antibodies may complicate the detection and identification of immune, clinically significant antibodies.

A clinically significant antibody can be defined as one capable of causing accelerated destruction of a significant proportion of transfused cells, or one capable of crossing the placenta and causing haemolytic disease of the fetus and newborn. When the alloantibodies combine with the corresponding RBC antigens, it can cause acute haemolytic transfusion reaction (AHTR) or delayed haemolytic transfusion reaction (DHTR) [9]. The specificity, Ig class, thermal range and concentration of the antibody can predict clinical significance; however, the clinical situation and patient's individual immune response are also significant factors [9]. The aim is to detect antibodies that are likely to cause problems in the clinical setting for which the antibody screening is designed, for example patient pretransfusion, antenatal or donor.

All immunoglobulins can be significant for transfusion medicine; however, IgG, IgM, and IgA have the most significance. Most clinically significant antibodies that react at body temperature (37°C) are IgG isotype and are capable of destroying transfused antigen-positive RBCs, causing anemia and transfusion reactions of various severities. IgM antibodies are most commonly encountered as *naturally* occurring antibodies in the ABO system and are believed to be produced in response to commonly occurring antigens like intestinal flora and pollen grains. Other blood groups such as Lewis, Ii, P, and MNS may also produce IgM antibodies, which usually react best at ambient temperature (22°C to 24°C). The primary testing problem encountered with IgM antibodies is that they can interfere with the detection of clinically significant IgG antibodies by masking their reactivity. Unlike IgG, IgM exists in both monomeric and polymeric forms (as pentamers) containing a J (joining) chain [9].

IgG antibodies are significant in transfusion medicine, because they are the class of immunoglobulins that are made in response to transfusion with nonself antigens on blood products. IgG antibodies are important in HDFN, because antibodies can be formed in response against allo-antigens on fetal RBCs that enter the mother's circulation, usually during delivery. Antibody screening, Rh typing, and passive anti-D antibody have prevented HDFN from developing in D-negative mothers who give birth to D-positive babies [10]. Fetal anemia in anti-Kell HDFN is associated with suppression of erythropoiesis due to destruction of erythroid precursor cells, which can be additional to destruction of circulating antigen-positive RBCs, as seen in anti-D HDFN. Serum IgA is found in both monomeric and polymeric forms; however, secretory IgA is usually found in the mucosal tissues of the body. Its polymer form acquires a glycoprotein secretory component as it passes through epithelial cell walls of mucosal tissues and appears in nearly all body fluids. Around 30% of anti-A and anti-B antibodies are of the IgA class and also, anti-IgA antibodies can cause severe anaphylaxis if IgA are transfused in plasma products to patients who are deficient in IgA. Another reason for the importance of IgA is that it can increase the effect of IgG induced RBC hemolysis [9].

Karl Landsteiner, the father of transfusion medicine discovered the first human blood group system, ABO in 1901. This marked the beginning of the concept of individual uniqueness defined by the RBC antigens present on the RBC membrane. For the next 45 years, only those antibodies that directly agglutinate red cells could be studied. With the development of the antiglobulin test by Coombs, Mourant, and Race in 1945, non-agglutinating antibodies could be detected and the science of blood group serology blossomed. Between 1946 and 1947 with the use of the indirect antiglobulin test the Kell (K), Duffy (Fy), Kidd (Jk), Diego (Di), Cartwright (Yt), Xg,

Dombrock (Do) and Colton (Co) blood group systems were discovered. The International Society of Blood Transfusion (ISBT) Working Party on Terminology for Red Cell Surface Antigens was set up in 1980 to establish a uniform nomenclature for all blood group antigens. Now 346 blood group antigens are identified and out of them, 308 are clustered within 36 blood groups systems. Remaining are accommodated in the Collections and Series: 700 Series of Low Incidence Antigens & 901 Series of High Incidence Antigens.

The AHG test/Coombs' test, is based on the principle that antihuman globulins obtained from immunized non-human species bind to human globulins such as IgG or complement, either free in serum or attached to antigens on red blood cells. Because of their large pentamer structure, IgM antibodies bind to corresponding antigen and directly agglutinate RBCs suspended in saline. Some IgG antibodies are termed non-agglutinating, or incomplete antibodies, because their monomer structure is too small to directly agglutinate sensitized RBCs. Adding AHG that contains anti-IgG to RBCs sensitized with IgG antibodies allows for hemagglutination of these sensitized cells. We use an anti-antibody to observe the formation of an Ag/Ab complex that would otherwise go undetected. Some blood group antibodies have the ability to bind complement to the RBC membrane. In such cases, an anti-complement component can be added to the AHG reagent, rendering it polyspecific. Antiglobulin tests detect IgG or complement-sensitized RBCs [9].

Apart from AHTR and DHTR along with HDFN, transfusion-related acute lung injury (TRALI) is the most severe adverse outcome from transfusion, with estimates of prevalence ranging from 0.02% to 0.16% of patients receiving transfusions [12,13] which is also an antibody mediated adverse reaction. TRALI is the clinical syndrome of new acute lung injury (ALI)

that develops with a clear temporal relationship to transfusion, in patients with or without alternate risk factors for ALI [14]. TRALI manifests as fever, hypoxemia, and pulmonary oedema occurring acutely (within 6 hours) following transfusion of blood components. The exact pathophysiology of TRALI is unclear, though TRALI is strongly associated with the presence of allotypic antibodies present in donor blood products. Antibodies against HLA class I and class II molecules have been found in 50% to 89% of products associated with TRALI. Additionally, antibodies to HNA-1a, HNA-1b, HNA-2a, and HNA-3a, allo-antigens found specifically on neutrophils, have been described in as many as 72% of blood products associated with TRALI [15,16]. The current model of TRALI suggests that these allotypic antibodies activate neutrophils in the lung, which release cytokine and chemokine mediators that cause pulmonary oedema [17]. The current models of the pathogenesis of TRALI secondary to the infusion of donor antibodies directed against recipient HLA class II antigens are based on associative data and require proinflammatory activation of the pulmonary vascular endothelium and circulating monocytes, resulting in polymorphonuclear neutrophil (PMN)-mediated endothelial damage, capillary leak, and ALI [18].

Detection of ‘insignificant’ antibodies creates additional work and leads to potential delay in providing compatible blood for transfusion, or unnecessary repeat testing/interventions in pregnancy. The balance between sensitivity and specificity can be influenced by the methods and technologies selected. It is not possible to detect all potentially clinically significant antibodies, or to avoid detecting all clinically insignificant antibodies, that is ‘noise’ [19]. Antibody detection (screening) can be optimized by designing protocols appropriate to the population to be screened, utilizing knowledge of the characteristics of red cell antibodies,

understanding of the principles, strengths and limitations of the methods/technologies validated for use, and ongoing quality assurance of testing. All alloantibodies detected in screening should be investigated for specificity, and the results interpreted in clinical context. Automation is happening in every arena of Transfusion Medicine and now we also have an automated system (software) to interpret specificity of allo-antibody. Such software uses an algorithm based on behaviour and characteristics of antibodies. Antibody identification software can support the technicians in identifying antibodies and can also guide to carry out supplementary tests to rule out other antibodies. It may be used as a supplement or may eventually replace manual method of antibody identification [20].

INCIDENCE OF UNEXPECTED ANTIBODIES IN DIFFERENT POPULATIONS

According to a study from New England Research Institute, only 0.3–1.0% of general population have unexpected antibodies and the incidence is higher in women due to the pregnancy and patients receiving multiple transfusions [21]. But generally, the prevalence of clinically significant alloantibodies has been reported from less than 0.3% to up to 60% of samples depending on the study populations and the test method sensitivity [22,23]. Not uncommonly, autoantibodies can also be found along with alloantibodies which have been reported to be as high as 28% [24].

A study from Middle east Kuwaiti population states that prevalence of alloimmunization among blood recipients and donors (without the inclusion of pregnant women) was 0.49%. In general, the five most frequently identified alloantibodies were anti-D (27.3%), anti-E (18.5%), anti-K (15.6%), anti-Le^a (8.7%) and anti-Le^b (6.6%) [25]. A study on

prevalence and specificities of red blood cell alloantibodies in transfused Ugandans with different diseases [26] states that out of 113 transfused patients, 13 developed RBC alloantibodies in which 11 patients produced a total of 12 RBC alloantibodies of known specificity and remaining two patients possessed pan-reactive antibodies. The specificities of the alloantibodies identified were: 6 patients developed anti-E, 3 got anti- S and 1 each of anti-D, -K and -Le^a. In one patient, two alloantibodies, anti-E plus anti-K, presented as a combination; the rest of the alloantibodies were as single specificities. In an Indian study, incidence of RBC alloimmunization in transfused patients was 3·4% (18/531), with anti-c being the most common specificity (38·8%), followed by anti-E (22·2%), anti-M (11·1%), anti-Le^a (11·1%), anti-D (5·6%), anti-Jk^a (5·6%) and anti-Le^b(5·6%) [27]. In Chinese population of Guangxi, a study on RBC alloimmunisation rates among 20283 hospitalised patients, 166 alloantibodies were identified in 150 (0·74%) patients. Of the 150 patients with alloantibodies, 134 (89·33%) had a single alloantibody, whereas the remaining 16 (10·67%) had multiple alloantibodies. Out of these antibodies, the five most frequently identified alloantibodies were anti-E (39·76%), anti-Mia /Mur (11·45%), anti-c (10·84%), anti-Le^a (8·43%) and anti-M (6·63%) [28].

From a Northern Indian study, the cumulative incidence of irregular antibodies amongst the patients and donors were found as 0.12% and 0.009%, respectively. The incidence (0.09%) of Rh antibodies was found to be highest among the clinically significant antibodies. Anti-D antibody was the commonest antibody in Rh blood group system and the second most common blood group associated with the relatively higher rate of antibody incidence was MNS blood group system. The preponderance of Rh antibodies is also because of absence of universal RhD prophylaxis in pregnant women in the country as a whole. However, amongst the blood

donors the commonest blood group system associated with highest incidence of clinically significant antibody was MNS [29]. The incidence of DAT amongst the healthy blood donors in this was 0.04%. In western literature, similar studies in 1977 & 1989 reported as 0.3% and 38% respectively [30,31]. The pioneer study report on incidence of irregular antibodies and DAT in northern Indian population is 0.009% & 0.41% respectively in blood donors. It does add a new perspective that the probability of an irregular antibody is around 13 times (0.009% vs. 0.12%) lower in a healthy donor as compared to a patient [29]. This also brings forth a paradigm that 4 out of 10,000 (0.04%) healthy donors can have DAT positive without any obvious clinical signs and symptoms of auto-immune hemolysis. It is this category of donor units which show up as 'incompatible' even when patient's type and screen is negative. Therefore, as an institutional policy, these units are discarded (because these units cause problems in the compatibility testing). There is also a need to do an elaborate long-term follow-up on these donors since there is an evidence of a significantly increased risk of cancer, especially hematologic malignancies, among blood donors with a positive DAT [29,32]. Another Indian study on prevalence of unexpected red cell antibodies in blood donor population revealed that 0.27% (227/82,153) were positive for antibody screening using pooled O cells [33].

In western literature, Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) by National Heart, Lung, and Blood Institute (NHLBI, Bethesda), indicates that of the 2,045,204 donations, 10,450 (0.5%) had a positive RBC allo-antibody screen. Of the 632,378 unique donors, 4,861(0.77%) had a positive antibody screen on one or more of their donations over the study interval. The distribution of antibody specificities varied by sex, with anti-D being more prevalent in females and anti-K being

more prevalent in males [34]. Another Colombian study on blood donors' states that during the period 2007–2009, there were 60,309 whole-blood donations, and 438 alloantibodies were found (0.73%), 66.7% of them in female blood donors and 33.3% in male donors. The clinically significant were 140 (31.96%) and non-clinically significant were 300 (68.5%). The descending order of prevalence were anti-Le^a, antibodies against low prevalence antigens, cold antibodies, non-specific IgG, anti-Le^b, anti-D, anti-E, anti-K and anti-M [35].

DAT POSITIVITY AMONG HEALTHY DONORS

The incidence of positive DATs amongst healthy blood donors with a normal haemoglobin level varies from 1 per 9,000 to 1 per 14,000 [36,37]. In the majority of case, long term follow-up of these donors reveals no major clinical sequelae and no apparent cause for DAT positivity. Consequently, the reason for the positive DATs cannot be explained satisfactorily [37].

Healthy individuals can have 5–90 IgG molecules/red cell [38] and 5–40 C3d molecules/red cell [39]; these levels are usually below the threshold of detection in routine testing. Depending on the technique and reagents

used, the DAT can detect 100–500 molecules of IgG/red cell and 400–1100 molecules of C3d/ red cell. Positive DATs are reported in 1:1000 up to 1:14,000 blood donors and 1–15% of hospitalised patients [40]. These large differences in incidence probably relate to the different techniques used for performing the test. Based on Glasgow and West of Scotland Blood Transfusion Service studies that serves a population of approximately three million people, the incidence of positive DAT among healthy blood donors

in the west of Scotland is approximately 1 per 7,000 donations [41]. But in some populations, positive DATs are related to intercurrent viral infections or existing immune disorders [42,43]. Positive DATs due to antiphospholipid antibodies (APAbs) have now been well described, both in the antiphospholipid syndrome with systemic lupus erythematosus and the primary antiphospholipid syndrome, the incidence being 16% in the former and 4.3% in the latter [44]. APAbs may occur in association with a variety of auto-immune disorders including systemic lupus erythematosus, malignancy or infection and, very occasionally in the apparently healthy [42]. In Glasgow and West Scotland study, they found that sixty-six units from 42 donors were found to be DAT positive and eight blood donors with positive DATs also had false positive VDRL tests out of 474,545 donations over 3 years [41]. Out of these eight donors, 3 had elevated APAbs also. The well-documented association of APAbs in the antiphospholipid syndrome with positive DATs led them to believe that the positive DATs in these 3 healthy blood donors (3 out of 42) was due to cross-reacting APAbs adsorbing non-specifically on to the erythrocyte membrane and binding to phospholipid epitopes. Therefore, APAbs may be an incidental cause of positive DATs amongst healthy blood donors.

However, a small number of donors, in particular, those with persistence of IgG on the RBCs, may be in the early stages of an autoimmune illness and may be developing a serum protein abnormality that could presage a more serious illness such as a malignancy or a hematologic disorder. As the opportunity exists for early diagnosis of a clinically significant and potentially treatable illness, the follow-up of blood donors with a positive DAT and IgG on the RBCs is recommended by most authors. If a donor is deferred based on a positive DAT, it is appropriate that they be referred to a physician for periodic assessment and follow-up [45,46]. Legal and

ethical standards recognize that a blood donor has the right to be provided with any information that may be significant for his or her health and that these are also important considerations in determining policies for management of blood donors in this situation.

CLINICALLY SIGNIFICANT RED CELL ANTIBODIES

Clinically significant red cell antibodies are usually IgG antibodies active at 37^0C and/or by the indirect antiglobulin test. They cause HDFN and HTRs by destroying the red cells and thereby reducing its lifespan and associated consequences. AHTR consists of acute hemolysis with accompanying presenting symptoms within 24 hours of transfusion which may be immune or non-immune origin. Previously formed IgM or IgG antibodies in the recipient recognize the corresponding donor red cell antigens, immune complexes are formed, the complement cascade is activated, vasoactive amines and inflammatory mediators are released into the plasma, and the coagulation cascade is activated [47]. The activated lytic arm (membrane attack complex) of the complement cascade causes hemoglobinemia and hemoglobinuria, the hallmarks of intravascular hemolysis. DHTR is classically caused by a secondary immune reaction where alloantibodies undetectable at the time of transfusion rebound following exposure to the corresponding RBC antigens, leading to the RBC destruction [48]. DHTR occurs between 3 and 22 days after RBC transfusion. The Immunohematology Reference Laboratory (IRL, formerly the Red Cell Reference Laboratory) has served the Pacific Northwest as a regional reference laboratory for over 30 years. IRL classifies red cell antibodies into Group I to Group IV. Group I is clinically significant antibodies which includes ABO, Rh (D, C, E, c, e), Kell, Duffy, Kidd, S

and s. Group II being benign antibodies mainly Chido/Rodgers (Ch^a/Rg^a), Xg^a, Bg, Cs^a, Kn^a, McC^a, Yk^a and JMH. Group III consists of clinically insignificant if not reactive at 37°C; possibly significant when reacting at 37°C (Lewis, MN, P, Lutheran and A_1) and Group IV have antibodies that are sometimes clinically significant (Yt^a, Vel, Ge, Gy^a, Hy, Sd^a). Only a few antibody specificities are commonly seen: M, P1, and I antibodies react at room temperature and are considered clinically insignificant; K, S, s, Fy^a, Fy^b, Jk^a, and Jk^b antibodies react in the antiglobulin phase and are clinically significant.

American Association of Blood Banking (AABB) states that if an antibody screen is positive, an attempt should be made to identify the antibody and cross match for the same along with ABO and Rh. If the antibody screen is negative, they recommend only ABO/Rh compatibility testing to be done before transfusion. However, there is great paucity of literature regarding the scenario where the antibody screen is positive but the antibody identification is inconclusive, to see if this has any clinical implications. As the literature states antibodies against Rh system especially Rh (D, C, E, c, e) is very common among general population because of absence of universal RhD prophylaxis in pregnant women in almost all developing and underdeveloped countries as a whole.

Most of the published literature refers to antibodies of Lewis blood group system to be insignificant, whereas antibodies to M and N blood groups are associated with variable clinical significance. Antibodies against MN and Lewis blood group antigens with their thermal amplitudes in the range of 22-30°C gain special importance in certain conditions of induced hypothermia. These antibodies with a higher thermal range, which would otherwise be termed clinically insignificant, will induce *in vivo* hemolysis in patients with lowered core body temperature, which is now a common

practice in various surgeries such as neuro surgeries, cardiac surgeries etc [49]. Therefore, the thermal amplitude of the antibody must always be determined and if judged to be clinically significant, corresponding antigen negative blood must be provided. Many examples of anti-M are naturally occurring saline agglutinins that react below 37°C. the antibody can have IgM and IgG components. They do not bind complement, regardless of their immunoglobulin class, and they do not react with enzyme-treated RBCs. Anti-M appears to be more common in children than in adults and is particularly common in patients with bacterial infections [50]. When anti-M reacts at 37°C, it is sufficient to provide units that are crossmatch-compatible at 37°C and at the antiglobulin phase without typing for M antigen. Anti-M rarely causes HTRs, decreased red cell survival, or HDFN.

Lewis antibodies are often naturally occurring and made by Le (a– b–) persons; that is, they occur without any known RBC stimulus. They are generally IgM and do not cross the placenta. Because of this and because the Lewis antigens are not well developed on fetal RBCs, the antibodies do not cause HDFN. Lewis antibodies occur quite frequently in the sera of pregnant women who transiently exhibit the Le (a– b–) phenotype. Anti-Le^a is the most commonly encountered of the Lewis antibodies and is often detected in room temperature tests, but it sometimes reacts at 37°C and in the indirect antiglobulin test. Rare hemolytic transfusion reactions have been reported in patients with anti-Lea who were transfused with Le(a+) RBCs, so anti-Lea that are reactive at 37°C, particularly those that cause in vitro hemolysis, should not be ignored [51].

In the twentieth century, Landsteiner [52] first described blood agglutination at cold temperatures and Schubothe named the disorder ‘‘cold agglutinin disease’’ [53] ‘Physiological’ cold agglutinins (CA) or benign cold agglutinins (BCAs) develop because of change in expression of red

cell antigens that occurs after birth. These antibodies react best at about 4^0C and are present in the serum of almost all individuals as low titre (<1:10) auto-antibodies [54]. Moderately, high titre CAs with higher thermal amplitude was associated with certain infections. For example, anti-I with Mycoplasma, Listeria, Leishmaniasis; anti-i with infectious mononucleosis, and anti-Pr with viral infections like rubella or Varicella [55]. Pathological cold agglutinins are maximally reactive at around 28–31^0C and tend to occur at very low titres [56]. In 90 % of cases, cold agglutinins are IgM by nature. Rarely, monoclonal IgG, IgA, or k light chain restriction, are responsible. CAs can interfere with blood grouping and cross-matching. It is necessary to use O reagent cells in the reverse grouping and also to carefully note the strength of the agglutination. In cases of severe disease, samples have to be collected and maintained at 37^0C until the plasma and red cells are separated or pre-warmed plasma (plasma kept at 37^0C for 10–15 min) may be used as was done in this case. For the DAT, the patient's red cells were washed with warm saline to remove residual plasma containing cold antibodies. Warming reduces the adsorption of the cold antibodies onto the red cells. Minimizing adsorption during sample processing yields more accurate titres of cold antibodies, and avoids false-positive results [57]. Spontaneous cold auto-agglutination phenomenon observed in blood units stored under blood bank conditions appears to be due to a presence of CA. As these auto-antibodies are strictly reactive at low temperatures, they do not give rise to in vivo hemolysis. As the antibody specificities identified are known to be associated with certain infections, their presence in healthy blood donors could be attributed to exposure to such infections in recent past or present in its subclinical phase at the time of blood donation [55].

Anti-I is a common autoantibody that can be found in virtually all sera, although testing at 4°C and/or against enzyme treated RBCs may be required to detect the reactivity [51]. Consistently strong agglutination with adult RBCs and weak or no agglutination with cord or adult i RBCs define its classic activity. Auto-anti-I, found in the serum of many normal healthy individuals, is benign—that is, not associated with in vivo RBC destruction. It is usually a weak, naturally occurring, saline-reactive IgM agglutinin with a titre less than 64 at 4°C. Stronger examples agglutinate test cells at room temperature and bind complement, which can be detected in the antiglobulin test if polyspecific reagents are used. Occasionally, benign cold auto-anti-I can cause problems in pretransfusion testing. Usually, avoiding room temperature testing and using anti-IgG instead of a polyspecific antihuman globulin help to eliminate detection of cold reactive antibodies that may bind complement at lower temperatures. Cold auto-adsorption to remove the autoantibody from the serum may be necessary for stronger examples; cold auto-adsorbed plasma or serum can also be used in ABO typing. Pathogenic auto-anti-I (e.g., the type associated with cold agglutinin syndrome) typically consists of strong IgM agglutinins with higher titres and a broad thermal range of activity, reacting up to 30° or 32°C. When peripheral circulation cools in response to low ambient temperatures, these antibodies attach in vivo and cause autoagglutination and peripheral vascular occlusion (acrocyanosis) or hemolytic anemia. The production of auto-anti-I may be stimulated by microorganisms carrying, I-like antigen on their surface e.g. *M pneumoniae* [9]. Many other I-related antibodies have been described: anti- IA, -IB, -IAB, -IH, -iH, -IP1, -ITP1, -IHLe^{b}, and -iHLe^{b} due to close relationship of I to the biochemical structures of ABH, Lewis, and P antigens. These specificities are not mixtures of separable antibodies; rather, both antigens must be present on

the RBCs for the antibody to react. Anti-IH is commonly encountered in the serum of group A1 individuals. Anti-IH reacts stronger with group O and group A2 RBCs than with group A1 RBCs. Anti- IH should be suspected when serum from a group A individual directly agglutinates all group O RBCs but is compatible with most group A donor units [9].

IMPORTANCE OF TYPE AND SCREEN

Several studies have proven that there is high incidence of irregular antibodies was in the patients following transfusion. In western literature as well as Indian studies the irregular antibody incidence range from 0.75% to 38% among these most of them were multi-transfused [23,27,58]. The least incidences indicate most of them followed Type and Screen strategy [59,60] than Type and Matching System which is routinely practiced in many places. There are two Indian studies [59,60] which have compared type and screen with conventional AHG cross-match. They have slightly different results. While the first study [60] had 45,373 patients receiving a total of 61,668 units of packed RBCs (PRBCs) which were cross-matched in the AHG phase (irrespective of the result of the antibody screen) with simultaneous antibody screen carried out in all the patients. They did not find a single case where the antibody screen was negative and AHG cross-match showed incompatibility. The study concluded that type and screen policy can be safe, efficient, cost-effective, and beneficial to the transfusion service in India. In the second study [59] on 2026 patients, one sample gave a negative antibody screen while conventional crossmatch was incompatible. The authors said that this may be due to a rare antibody in the patient sera against which the corresponding antigen was not present on the screening cells but present on the donor red cell. They said that a

transfusion reaction would have occurred in this case since it was reacting at 37^0C in AHG. The authors opined that since, the screening panel used in the present study was not from Indian sub-continent (panels are from Caucasian donors); therefore, it would be advisable to prepare screening cell panels that include RBC antigens which are prevalent within the local/regional population e.g., In, Mi^a etc.

MATERIALS AND METHODS

Using a cross sectional study design, randomly chosen 7000 voluntary donors coming for blood donation during a period of 1 year from Dec 2017 to Dec 2018 were screened for presence of unexpected red cell antibodies. Donors were screened according to departmental as well as DGHS guidelines and were given prescribed questionnaires to fill up. Later they underwent counselling and a medical examination as a part of blood donor assessment. Eligible donors underwent phlebotomy and after blood donation, blood samples were collected for routine immunohematological investigations, Transfusion Transmitted Infections (TTI) screening and antibody screening as per prescribed DGHS guidelines. For this study, an additional 2ml of blood was collected in EDTA test tube for performing DAT, along with pilot test tubes.

All donors were screened for red cell antibodies by Direct Antiglobulin Test and Indirect Antiglobulin Test primarily along with routine 'O' papanisation during reverse grouping. In DAT, donor cells are screened and in IAT donor serum is used for antibody detection. DAT positivity indicates in vivo sensitisation as well as IAT indicates in vitro sensitisation of the donor with an antibody. DAT and IAT after incubation at 37^0C positive blood unit will be discarded as hospital blood transfusion policy. Auto-control using donor cells and serum is required for checking the validity of the test as well as confirmation of auto-agglutinins after incubation of the same at different temperatures (4^0C, 22 ^{0}C & 37^0C).

If Direct Antiglobulin Test is found positive, then further antibody determination is done by different types of Elution methods namely heat elution and ether elution techniques. If IAT is positive, then antibody screening and identification is done using red cell panels. Antibody titration also follows identification procedures. Secretor status was also performed

in 3 donors with anti-IH and anti-Lewis antibodies. These antiglobulin test positive donors were called back for notification and another fresh sample were taken for retesting and given proper counselling as well as follow up of the donor was also performed. My thesis concentrated on healthy voluntary donor population and later on similar study can be extended out to patient population to get a better prevalence among general population.

TESTING FOR ABNORMAL RED CELL ANTIBODIES APART FROM TESTING PAPAINISED O POOLED CELLS ALONG WITH REVERSE GROUPING

(Adopted from AABB Technical Manual 18th edition; USA 2014)

METHOD 1: PREPARING A 3-5% RED CELL SUSPENSION

Principle: A 3% red cell suspension is a common reagent in many serologic procedures. This achieves the appropriate serum-to-cell ratio for most test procedures and for an adequate number of red cells so one can read and grade the reactions.

Materials

1. EDTA whole blood sample
2. Test tubes
3. Glass Pauster pipettes (5-mL serologic)
4. 0.9% Normal saline (NS)
5. Table top centrifuge (3000 rpm or equivalent)
6. Laboratory prepared 3% pooled O red cell suspension

Procedure:

Step	Action
1	Transfer at least 1 mL of whole blood to a 10-mL tube
2	Wash the red cells in saline, centrifuging for 5 minutes to pellet the cells. Repeat two or three times. The final supernatant should be clear and should be completely removed by aspiration
3	Transfer 0.3 mL of the washed red cells to a tube with 9.7 mL of saline and thoroughly mix the red cells and saline by gently inverting the tube several times
4	Label the tube as 3% donor cell suspension with donor ID number

METHOD 2: SALINE INDIRECT ANTIGLOBULIN TEST

PROCEDURE

Principle: An indirect antiglobulin test demonstrates in-vitro reactions between red cells and antibodies, and is used in antibody detection, antibody identification, crossmatching, and blood group phenotyping. The saline method is performed when red cells are washed to remove unbound globulins.

Specimen: Serum is used from clotted pilot tube

Reagents:

1. Normal saline
2. Polyspecific and monospecific Antihuman globulin (AHG) reagent
3. 3-5% pooled group O cell suspension
4. A 3-5% suspension of donor red cells in saline after 3 times washing in normal saline
5. IgG-coated red cells

Procedure:

Step	**Action**
1	Add 1 drops of donor serum to properly labelled tubes
2	Add 1 drop of 3-5% saline-suspended reagent group O red cells and 3-5% donor red cell saline suspension (auto-control) to each tube and mix
3	Centrifuge and observe for hemolysis and agglutination. Grade and record the results
4	Incubate at 37^0C for 60 minutes
5	Centrifuge and observe for hemolysis and agglutination. Grade and record the results

6	Wash the red cells three times with saline, and completely decant the final wash
7	Add 1 drop polyclonal AHG to the dry red cell button. Mix well and then centrifuge immediately at 1000 rpm for 1 minute.
8	Observe for agglutination. Grade and record the results. Confirm the validity of negative results by adding IgG-coated red cells to the supernatant.

Interpretation:

1. The presence of agglutination/hemolysis after incubation at 37^0C constitutes a positive test result.
2. The presence of agglutination after addition of polyclonal AHG constitutes a positive test result. Test with monoclonal AHG for specificity.
3. Antiglobulin test results are negative when no agglutination is observed after initial centrifugation followed by agglutination with the addition of IgG-coated red cells and centrifugation. If the IgG-coated red cells are not agglutinated, the negative result is invalid and the test must be repeated.

METHOD 3: PERFORMING A DIRECT ANTIGLOBULIN TEST

Principle: The direct antiglobulin test (DAT) can determine if red cells have been coated in vivo with immunoglobulin, complement, or both. It is used primarily for the investigation of hemolytic transfusion reactions, hemolytic disease of the fetus and newborn, autoimmune hemolytic anemia, and drug-induced immune hemolysis.

Specimen: Red cells from an EDTA-anticoagulated blood sample

Reagents:

1. Antihuman globulin (AHG) reagent: polyspecific antiglobulin reagent, anti-IgG, anticomplement antisera
2. A control reagent (e.g., saline or 6% albumin) is required when all antisera tested give a positive result
3. IgG-coated red cells.

Procedure:

Steps	Action
1	Dispense 1 drop of 3- 5% suspension of donor red cells into a tube for each antiglobulin reagent or control to be tested
2	Immediately add polyspecific AHG and mix followed by centrifugation at 1000 rpm for 1 minute
3	Examine the cells for agglutination. Grade and record the reaction

4	Confirm the specificity by testing with monoclonal AHG
5	Add IgG-coated red cells to test supernatant containing anti-IgG to validate the negative result

Interpretation:

1. The DAT is positive when agglutination is observed either after immediate centrifugation or after the centrifugation that followed room-temperature incubation. IgG-coated red cells usually give immediate reactions, whereas complement coating may be more easily demonstrable after incubation. Monospecific AHG reagents are needed to confirm which globulins are present.
2. The DAT is negative when no agglutination is observed at either test phase and the IgG-coated cells are agglutinated. If the IgG-coated cells are not agglutinated, the negative DAT result is considered invalid, and the test must be repeated. A negative DAT does not necessarily mean that the red cells have no attached globulin molecules. Polyspecific and anti-IgG reagents detect 150 to 500 molecules of IgG per cell, but patients may still experience autoimmune hemolytic anemia when IgG coating is below this level.
3. No interpretation can be made if the control reagent is reactive. This may indicate the presence of a strong cold auto-agglutinin or spontaneous agglutination due to warm reactive IgM or IgG antibodies. Warming the red cells to 37^0C and/or washing with warm (37^0C) saline should resolve reactivity due to cold auto-agglutinins.

METHOD 4: AUTO-CONTOL AND SERUM WITH POOLED O CELLS INCUBATION AT LOWER TEMPERATURES FOR WEAK AGGLUTININS

Principle: Prolonged incubation at low temperatures can enhance antibody binding and detection of IgM antibodies in the donor serum.

Specimen:

1) washed 3-5% donor red cell suspension

2) Donor serum

Reagents:

1) Kahn tubes

2) Washed 3-5% pooled O cells

Procedure:

Steps	Action
1	Add one drop of donor serum to 4 test tubes labelled A, B, C & D
2	Now add one drop of donor cells to each of test tubes A & B and add one drop of pooled O cells to tubes C & D
3	Now incubate tubes A & C at room temperature and tubes B & D at 4^0C for 30 minutes
4	Centrifuge tubes at 1000rpm and gently resuspend cell buttons and examine for agglutination

Interpretation:

1. Tubes A and B are auto-controls at 22^0C and 4^0C respectively & positivity indicates presence of auto-agglutinins or benign cold agglutinins (IgM) which are enhanced by cold temperatures.
2. Tubes C and D with pooled O cells at 22^0C and 4^0C respectively give positive reaction if IgM auto/allo-agglutinins or benign cold agglutinins that are present in the donor serum.
3. Normally benign cold antibodies present no serologic problem because routine antibody detection tests are not performed at this temperature. The typical cold agglutinin has a relatively low titre (less than 64 at 4°C). Occasionally, these benign cold autoantibodies may agglutinate cells at room temperature (20°C to 24°C); however, even in this situation, strongest reactivity is found at 4°C

METHOD 5: <u>ANTIBODY SCREENING USING 3 CELL PANEL</u>

Principle: The RBC reagents used in the antibody screen come from group 'O' individuals who have been typed for the most common, and the most significant, RBC antigens. Group O cells are used so that anti-A and anti-B will not interfere in the detection of antibodies to other blood group systems.

Specimens: 1) donor serum

Reagents:

1. 3% RBC reagent screening cells (SC) with 3 cell profile
2. Polyclonal AHG reagent
3. Saline

Procedure:

Steps	Action
1	Label 3 test tubes with proper labelling and add 1 drop of donor serum to each to the test tubes 1, 2 & 3
2	Add one drop of SC I to test tube 1, SC II to test tube 2 and SC III to test tube 3
3	Spin immediately at 1000rpm for 1 minute and look for agglutination by gentle resuspension of cell button
4	Now incubate the test tubes at 37^0C for 1 hour
5	Centrifuge and observe for hemolysis and agglutination. Grade and record the results
6	Wash the red cells three times with saline, and completely decant the final wash
7	Add 1 drop polyclonal AHG to the dry red cell button. Mix well and then centrifuge immediately at 1000 rpm for 1 min
8	Observe for agglutination. Grade and record the results. Confirm the validity of negative results by adding IgG-coated red cells to the supernatant.

Interpretation:

1. The presence of agglutination/hemolysis after incubation at 37^0C constitutes a positive test result.
2. The presence of agglutination after addition of anti-IgG constitutes a positive test result.

3. Antiglobulin test results are negative when no agglutination is observed after initial centrifugation followed by agglutination with the addition of IgG-coated red cells and centrifugation. If the IgG-coated red cells are not agglutinated, the negative result is invalid and the test must be repeated.
4. The antigram is used to conclude the possible most clinically significant antibodies.

Note:

1. The screen cells are packaged in sets of two or three cell suspensions, each having a unique combination of antigens. Within the set, there should be one cell that is positive for each of the following antigens: D, C, c, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P_1, M, N, S, and s.
2. Each set of screen cells will be accompanied by an antigen profile sheet,
   detailing which antigens are present in each vial of cells. These profiles are lot-specific and should not be interchanged.
3. There will be homozygous expression of many of the antigens within the screen cell set, allowing for detection of antibodies that show dosage (e.g., Kidd system), making it more reliable in detecting weakly reacting antibodies.

METHOD 6: ANTIBODY IDENTIFICATION USING 11 RED CELL REAGENT PANEL

Principle: An antibody identification panel is a collection of 11 to 20 group O RBCs with various antigen expression. The pattern of antigen expression should be diverse so that it will be possible to distinguish one antibody from another and should include cells with homozygous expression of Rh, Duffy, Kidd, and MNSs antigens.

Specimen: 1) Donor serum

Reagents:

1) 3% RBC reagent cells with 11 cell profile
2) Polyclonal AHG reagent
3) Saline

Procedure:

Steps	Action
1	Label 3 test tubes with proper labelling and add 1 drop of donor serum to each to the test tubes 1, 2 & 3
2	Add one drop of SC I to test tube 1, SC II to test tube 2 and SC III to test tube 3
3	Spin immediately at 1000rpm for 1 minute and look for agglutination by gentle resuspension of cell button
4	Now incubate the test tubes at 37^0C for 1 hour
5	Centrifuge and observe for hemolysis and agglutination. Grade and record the results
6	Wash the red cells three times with saline, and completely decant the final wash

7	Add 1 drop polyclonal AHG to the dry red cell button. Mix well and then centrifuge immediately at 1000 rpm for 1 minute.
8	Observe for agglutination. Grade and record the results. Confirm the validity of negative results by adding IgG-coated red cells to the supernatant.

Interpretation:

1. The presence of agglutination/hemolysis after incubation at 37^0C constitutes a positive test result.
2. The presence of agglutination after addition of anti-IgG constitutes a positive test result.
3. Antiglobulin test results are negative when no agglutination is observed after initial centrifugation followed by agglutination with the addition of IgG-coated red cells and centrifugation. If the IgG-coated red cells are not agglutinated, the negative result is invalid and the test must be repeated.
4. The antigram is used to solve the antibodies under question by application of different principles related to ruling in and ruling out possible antibodies

Note:

1. The pattern of antigen expression in the 11cell panel should be diverse so that it will be possible to distinguish one antibody from another and should include cells with homozygous expression of Rh, Duffy, Kidd, and MNSs antigens.

2. As with the screen cells, the profile sheet is lot-specific and should not be interchanged with that of another panel. The profile sheet will indicate the presence of rare cells, which are positive for low-prevalence antigens or negative for high-prevalence antigens.

METHOD 7: USING SULFHYDRYL REAGENTS TO DISTINGUISH IgM FROM IgG ANTIBODIES

Principle: Treating IgM antibodies with sulfhydryl reagents abolishes both agglutinating and complement-binding activities. Observations of antibody activity before and after sulfhydryl treatment are useful in determining immunoglobulin class. Sulfhydryl treatment can also be used to abolish IgM antibody activity to permit detection of coexisting IgG antibodies.

Specimen: 2 mL of serum to be treated

Reagents:

1. Phosphate-buffered saline (PBS) at pH 7.3.
2. 0.01 M dithiothreitol (DTT) prepared by dissolving 0.154 g of DTT in 100 mL of pH 7.3 PBS. Store at -18^0C or lower.

Procedure:

Steps	Action
1	Dispense 1 mL of serum or plasma into each of two test tubes
2	To one tube (labeled dilution control), add 1 mL of pH 7.3 PBS
3	To the other tube (labeled test), add 1 mL of 0.01 M DTT.

4	Mix and incubate at 37^0C for 30 to 60 minutes
5	Test the DTT-treated and dilution control samples in standard procedures

Interpretation:

1. Reactivity in the dilution control serum and no reactivity in the DTT-treated serum indicates an IgM antibody.
2. Reactivity in the dilution control serum and in the DTT-treated serum indicates an IgG antibody or an IgG and IgM mixture. Titration studies may be necessary to distinguish between them (see table below).
3. No reactivity in the dilution control serum indicates dilution of weak antibody reactivity and an invalid test.

Effect of Dithiothreitol on Blood Group Antibodies

Reciprocal of Serum Dilution						
Test Sample	**2**	**4**	**8**	**16**	**32**	**Interpretation**
Serum + DTT	3+	2+	2+	1+	0	IgG
Serum + PBS	3+	2+	2+	1+	0	
Serum + DTT	0	0	0	0	0	IgM
Serum + PBS	3+	2+	2+	1+	0	
Serum + DTT	2+	1+	0	0	0	IgG + IgM
Serum + PBS	3+	2+	2+	1+	0	

Control: A serum or plasma sample known to contain an IgM antibody should be treated and tested in parallel

Notes:

1. 2-mercaptoethanol can also be used for this purpose.
2. Sulfhydryl reagents used at low concentration may weaken antigens of the Kell system. For investigation of antibodies in the Kell system, it may be necessary to use other methods.
3. Gelling of a serum or plasma sample may be observed during treatment with DTT. This gelling can occur if the DTT has been prepared incorrectly and has a concentration above 0.01 M. Gelling may also occur if serum and DTT are incubated too long. An aliquot of the sample undergoing treatment can be tested after 30 minutes of incubation; if the activity thought to be caused by IgM has disappeared, there is no need to incubate further. Gelled samples cannot be tested for antibody activity because overtreatment with DTT causes the denaturation of all serum proteins.

METHOD 8: ANTIBODY TITRATION PROCEDURE

Principle: Titration is a semiquantitative method used to determine the concentration of antibody in a serum sample or to compare the strength of antigen expression on different red cell samples. The usual applications of titration studies are as follows:

1) estimating antibody activity in allo-immunized pregnant women to determine whether and when to perform more complex invasive investigation of the fetal condition
2) elucidating autoantibody specificity
3) characterizing antibodies as having high titre and low avidity, traits common in antibodies to antigens of the Knops and

Chido/Rodgers systems, Cs^a, and JMH; and 4) observing the effect of sulfhydryl reagents on antibody behaviour, to determine immunoglobulin class (IgG or IgM).

Specimen: Donor serum with abnormal antibody

Reagents:

1. Red cells that express the antigen(s) corresponding to the antibody specificity(ies), in a 2% to 5% saline suspension. Uniformity of red cell suspensions is very important to ensure comparability of results
2. Saline for dilutions

Procedure:

Steps	**Action**
	The master dilution technique for titration studies is as follows:
1	Label 10 test tubes according to the serum dilution (e.g., 1:1, 1:2, etc). A 1:1 dilution means one volume of serum undiluted; a 1:2 dilution means one volume of serum in a final volume of two, or a 50% solution of serum in the diluent.
2	Deliver one volume of saline to all test tubes except the first (undiluted, 1:1) tube.
3	Add an equal volume of serum to each of the first two tubes (undiluted and 1:2).

4	Using a clean pipette, mix the contents of the 1:2 dilution several times, and transfer one volume into the next tube (the 1:4 dilution).
5	Continue the same process for all dilutions, using a clean pipette to mix and transfer each dilution. Remove one volume of diluted serum from the final tube, and save it for use if further dilutions are required.
6	Label 10 tubes for the appropriate dilutions.
7	Using separate pipettes for each dilution, transfer 2 drops of each diluted serum into the appropriately labelled tubes, and add 2 drops of a 2% red cell suspension. Alternatively, for convenience, add 1 drop of a 3% to 4% suspension of red cells as supplied by the reagent manufacturer, although this method is less precise.
8	Mix well and test by a serologic technique appropriate to the antibody
9	Examine test results macroscopically; grade and record the reactions. The prozone phenomenon may cause reactions to be weaker in the more concentrated serum preparations than in higher dilutions. If one is to avoid misinterpretation of results, it may be preferable to examine first the tube containing the most dilute serum and then to proceed through the more concentrated samples to the undiluted specimen.

Interpretation:

1. Observe the highest dilution that produces 1+ macroscopic agglutination. The titre is reported as the reciprocal of the dilution level (e.g., 32—*not* 1 in 32 or 1:32). If there is agglutination in the tube containing the most dilute serum, the endpoint has not been reached, and additional dilutions should be prepared and tested.
2. In comparative studies, a significant difference in titre is three or more dilutions. Variations in technique and inherent biologic variability can cause duplicate tests to give results that differ by one dilution in either direction. Serum containing antibody at a true titre of 32 may show, on replicate tests, the endpoint in the 1:32 tube, the 1:64 tube, or the 1:16 tube.
3. Titre values alone can be misleading if the strength of agglutination is not also evaluated. The observed strength of agglutination can be assigned a number, and the sum of these numbers for all tubes in a titration study represents the score, another semiquantitative measurement of antibody reactivity. The arbitrarily assigned threshold for significance in comparing scores is a difference of 10 or more between different test samples.
4. Antibodies with high-titre and low-avidity characteristics generally have a titre greater than 64, with most tubes showing consistently weak reactivity.
5. The table below shows the results obtained with three sera, each of which shows no more agglutination after 1:256 dilution. The differences in score, however, indicate considerable variation in strength of reactivity.

Examples of Antibody Titres, Endpoints, and Scores

Reciprocal of Serum Dilution													
		1	2	4	8	16	32	64	128	256	512	Titre	Score
Sample 1	Strength	3+	3+	3+	2+	2+	2+	1+	±	±	0	64 (256)	
	Score	10	10	10	8	8	8	5	3	2	0		64
Sample 2	Strength	4+	4+	4+	3+	3+	2+	2+	1+	±	0	128 (256)	
	Score	12	12	12	10	10	8	8	5	3	0		80
Sample 3	Strength	1+	1+	1+	1+	±	±	±	±	±	0	8 (256)	
	Score	5	5	5	5	3	3	3	2	2	0		33

METHOD 9: DETERMINING THE SPECIFICITY OF COLD-REACTIVE AUTOAGGLUTININS

Principle: Cold-reactive auto-agglutinins are usually IgM, which binds to red cells in the lower temperature of the peripheral circulation and causes complement components to attach to the red cells. As the red cells circulate to warmer areas, the IgM dissociates but the complement remains.

Specimen:

1. Serum separated at 37^0C from a blood sample maintained and/or allowed to clot at 37^0C, or plasma, separated from an anticoagulated sample after periodic inversion at 37^0C for approximately 15 minutes.
2. Autologous red cells.

Reagents:

Test red cells of the following phenotypes:

1. A pool of two or more examples of adult group O I adult red cells; these can be the reagent cells routinely used for alloantibody detection
2. Group O i cord red cells
3. The patient's own (autologous) red cells, washed at least three times with 37^0C saline
4. Red cells of the same ABO group as the patient, if the patient is not group O. If the patient is group A or AB, use both A1 and A2 cells
5. Saline or phosphate-buffered saline (PBS), pH 7.3.

Procedure:

Steps	**Action**
1	Prepare serial two-fold dilutions of the serum or plasma in saline or PBS. The dilution range should be from 1 in 2 to 1 in 4096 (12 tubes), and the volumes prepared should be more than the total volume needed to test all of the desired red cells.
2	Label a set of 12 tubes with the dilution (e.g., 2, 4, 8, etc) for each kind of red cells to be tested (e.g., adult, cord, autologous).
3	Dispense 2 drops of each dilution into the appropriate tubes.
4	Add 1 drop of a 3% to 5% saline suspension of each red cell sample to the appropriate set of tubes.
5	Mix and incubate at room temperature for 30 to 60 minutes.

6	Centrifuge for 15 to 20 seconds at 1000 rpm. Examine the tubes one by one macroscopically for agglutination, starting with the set of tubes at the highest dilution for each cell tested (i.e., read all the tubes for each dilution as a set). Grade and record the results.
7	Incubate the tubes at 4^0C for 1 to 2 hours
8	Centrifuge for 15 to 20 seconds at 1000 rpm. Immediately place the tubes in a rack in an ice water-bath. Examine the tubes as in step 6. Grade and record the results.

Interpretation:

Typical Relative Reactivity Patterns of Cold Autoantibodies

Antibody Specificity					
Red cell	**Anti-I**	**Anti-i**	**Anti-I^T**	**Anti-IH**	**Anti-Pr**
O I adult	+	0/↓	0/↓	+	+
O i cord	0/↓	+	+	↓	+
A1 I adult	0/↓	+	0/↓	↓	+
A1 I cord	0/↓	+	+	↓	+
O I enzyme treated	+	0/↓	0/↓	↓	+
Autologous	↑	↑	↑	↑	0

+: reactive; 0: nonreactive; ↓: weaker reaction; ↑: stronger reaction

Agglutination Chart

Negative (0)

1+

2+

3+

4+

RESULTS

The sample size chosen for this study was 7000 healthy non-remunerated voluntary donors who were randomly chosen over a period between 26th November 2017 and 15th February 2019. Among the total subjects 5046 (72.08%) were males and 1954 (27.2%) were females (Figure 1). The age-wise distribution based on gender is shown in Graph 1. The males are more among all age groups and the number of blood donors are more in 18-30 years category and minimal donors below 50 years of age.

Among the 7000 samples analysed only 7 cases were positive for clinically significant irregular red cell antibodies. Hence the prevalence of alloimmunisation in healthy voluntary donor population in current study is 1 per 1000. There was no case of DAT positive in this study.

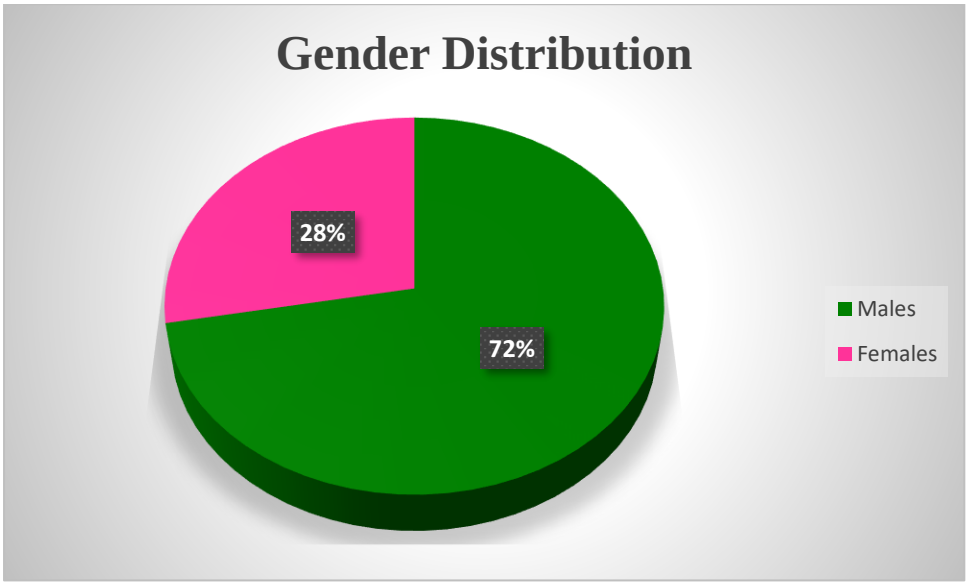
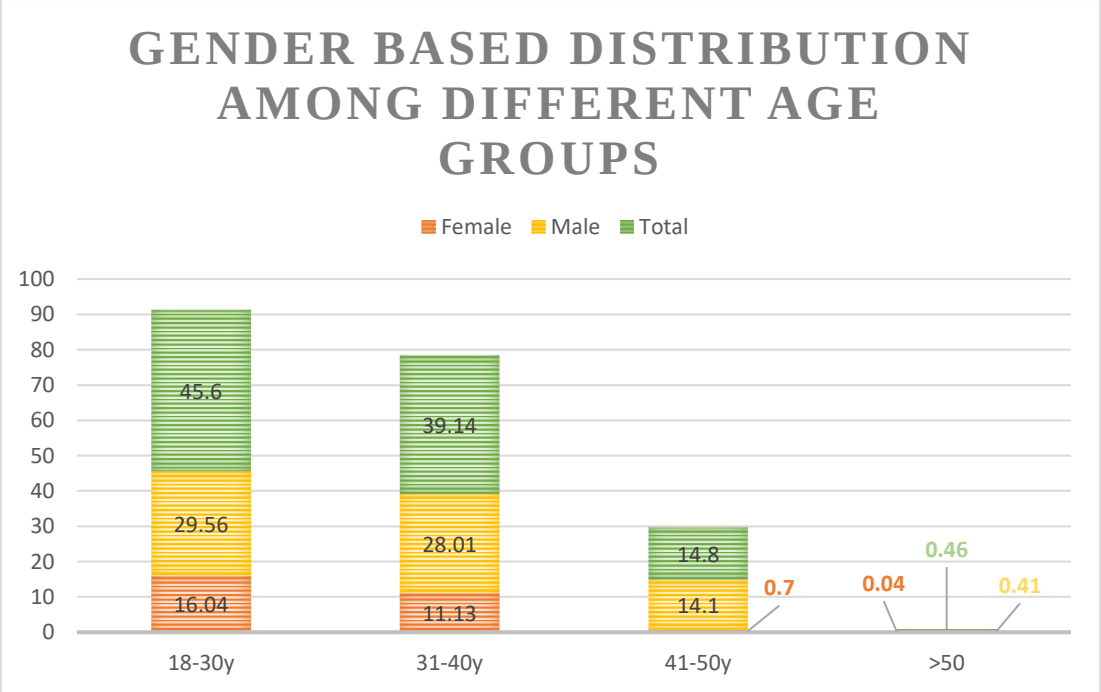


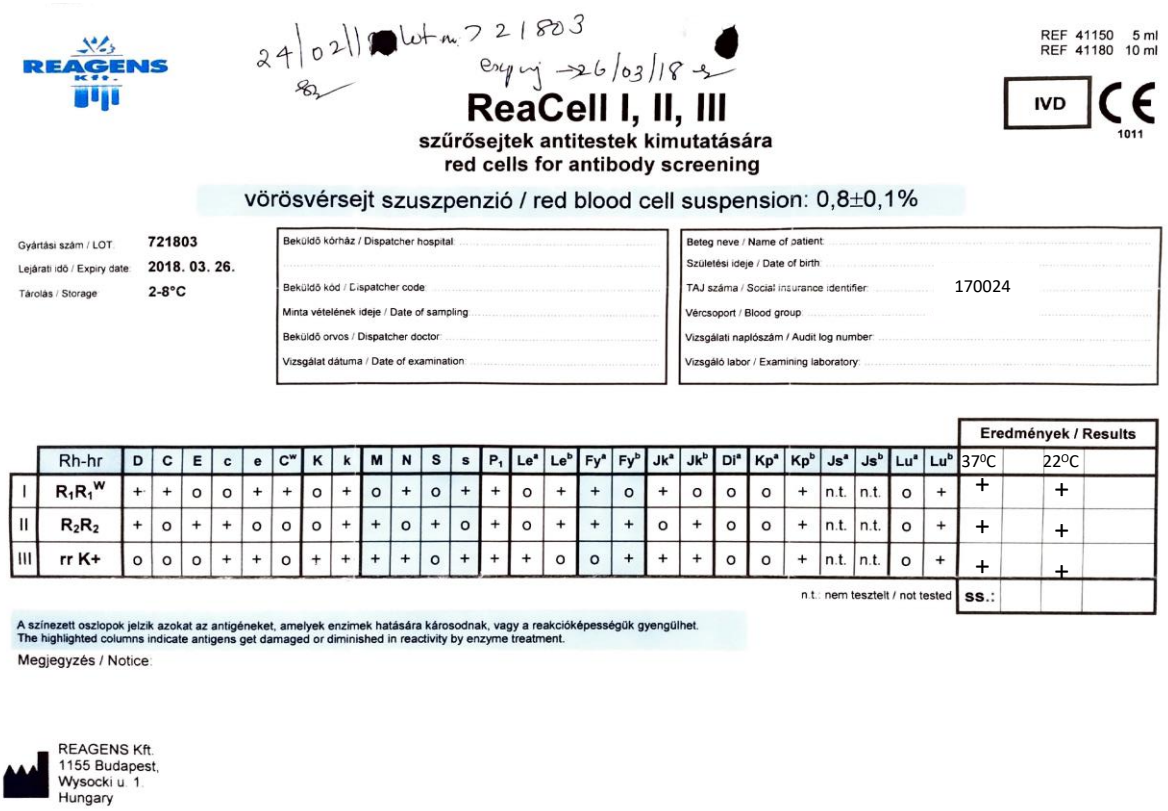
Figure 1



Graph 1

Out of the 7 positive cases of abnormal red cell antibodies, 2 cases were anti-M (28.57%), 2 cases were anti-Lewis (28.57%), 1 case of anti-D, 1 case of anti-C, 1 case of anti-IH. The cross-fabulation or testing the prevalence with respect to age group or sex is not possible because of this very low prevalence value. The details of the 7 cases that showed unexpected reactions are as follows:

Case 1: A 19-year-old male, first time donor, A negative blood group, with no previous history of previous history of transfusion, found to be having reaction with O cells and papanised O cells on reverse grouping. He had 2+ agglutination at 37^0C a with a negative DAT and auto-control at 37^0C and 22^0C. There was 4+ reaction with A1 lectin and no reaction with anti-H lectin. He had a positive auto-control at 4^0C. Pan-reactivity observed on 3 cell screening panel and 11 cell panel at 37^0C. There was no reaction observed with washed A positive and negative cells and washed A positive and negative cord cells.



REAGENS Kft.

24/02/[redacted] Lot no. 721803 exp. uj → 26/03/18-ig

REF 41150 5 ml
REF 41180 10 ml

IVD CE 1011

ReaCell I, II, III

szűrősejtek antitestek kimutatására
red cells for antibody screening

vörösvérsejt szuszpenzió / red blood cell suspension: 0,8±0,1%

Gyártási szám / LOT: **721803**
Lejárati idő / Expiry date: **2018. 03. 26.**
Tárolás / Storage: **2-8°C**

Beküldő kórház / Dispatcher hospital:
Beküldő kód / Dispatcher code:
Minta vételének ideje / Date of sampling:
Beküldő orvos / Dispatcher doctor:
Vizsgálat dátuma / Date of examination:

Beteg neve / Name of patient:
Születési ideje / Date of birth:
TAJ száma / Social insurance identifier: 170024
Vércsoport / Blood group:
Vizsgálati naplószám / Audit log number:
Vizsgáló labor / Examining laboratory:

	Rh-hr	D	C	E	c	e	C^w	K	k	M	N	S	s	P_1	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Di^a	Kp^a	Kp^b	Js^a	Js^b	Lu^a	Lu^b	Eredmények / Results 37°C	22°C
I	$R_1R_1^W$	+	+	o	o	+	+	o	+	o	+	o	+	+	o	+	+	o	+	o	o	o	+	n.t.	n.t.	o	+	+	+
II	R_2R_2	+	o	+	+	o	o	o	+	+	o	+	o	+	o	+	+	+	o	+	o	o	+	n.t.	n.t.	o	+	+	+
III	rr K+	o	o	o	+	+	o	+	+	+	+	o	+	+	+	o	o	+	+	+	o	o	+	n.t.	n.t.	o	+	+	+
																												ss.:	

n.t.: nem tesztelt / not tested

A színezett oszlopok jelzik azokat az antigéneket, amelyek enzimek hatására károsodnak, vagy a reakcióképességük gyengülhet.
The highlighted columns indicate antigens get damaged or diminished in reactivity by enzyme treatment.

Megjegyzés / Notice:

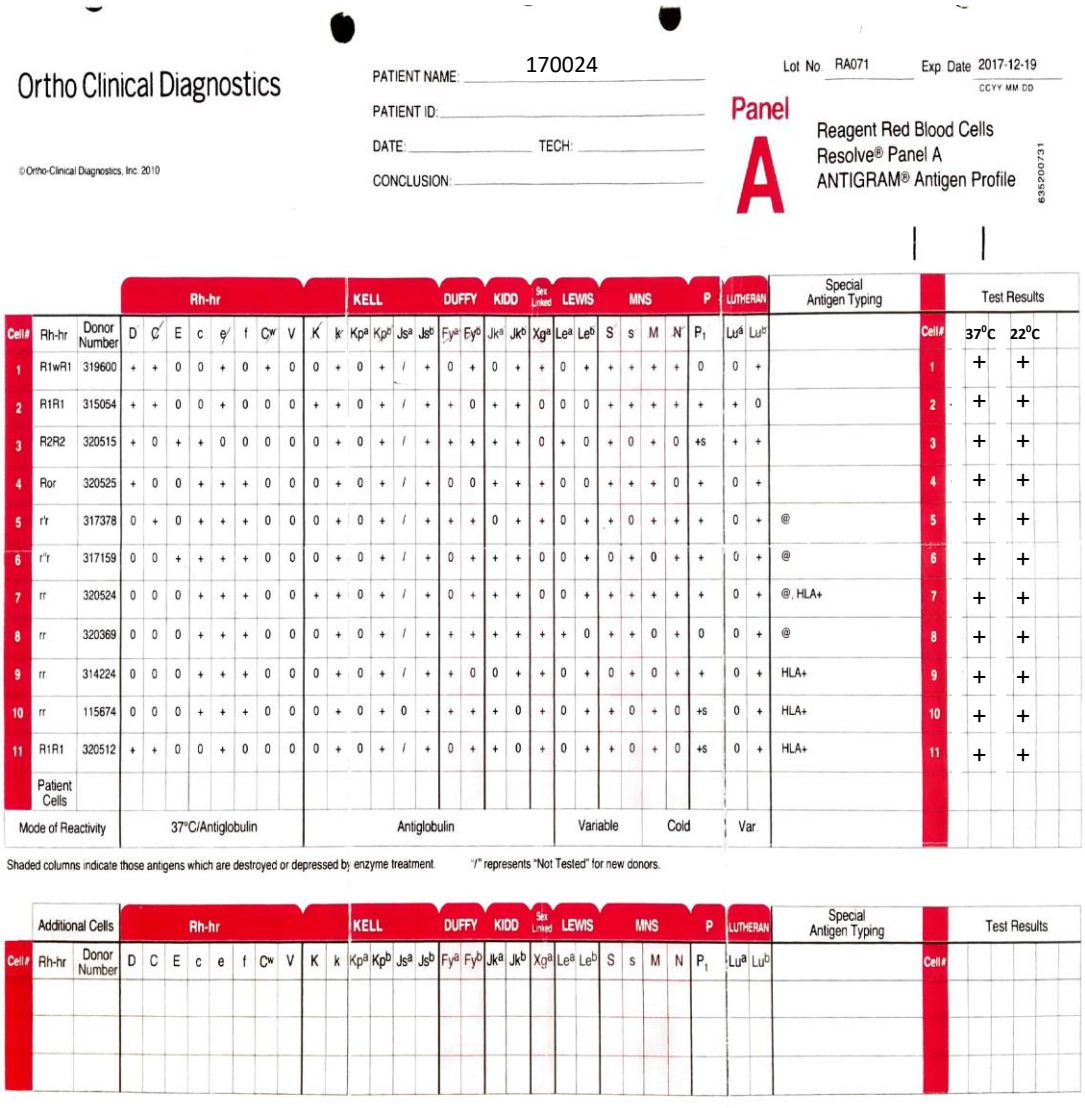
REAGENS Kft.
1155 Budapest,
Wysocki u. 1.
Hungary

Cells	O+	O-	A+	A-	O+ cord	O- cord	A+ cord	A- cord
37^0C	+2	+2	0	0	+wk	+wk	0	0
22^0C	+2	+2	0	0	0	0	0	0

Titre at 22^0C: 64

Titre at 37^0C: 32

After DTT treatment of serum, IgG titre: 2.



Ortho Clinical Diagnostics

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PATIENT NAME: 170024

PATIENT ID:

DATE: TECH:

CONCLUSION:

Lot No RA071 Exp Date 2017-12-19 CCYY MM DD

Panel A

Reagent Red Blood Cells
Resolve® Panel A
ANTIGRAM® Antigen Profile

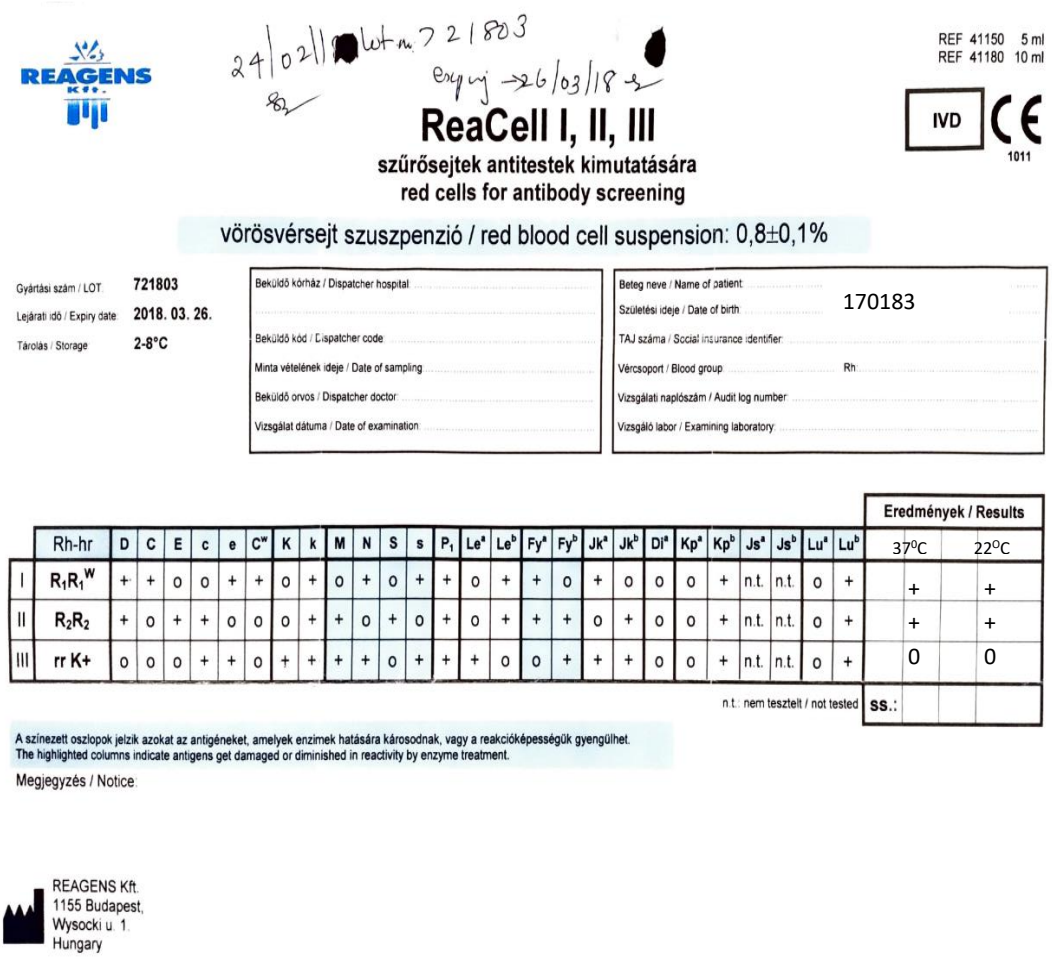
6352007731

			Rh-hr									KELL				DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing	Test Results	
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell #	37°C	22°C
1	R1wR1	319600	+	+	0	0	+	0	+	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	+	0	0	+		1	+	+
2	R1R1	315054	+	+	0	0	+	0	0	0	+	+	0	+	/	+	+	0	+	+	0	0	0	+	+	+	+	+	+	0		2	+	+
3	R2R2	320515	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	+	+	+	0	+	0	+	0	+	0	+s	+	+		3	+	+
4	Ror	320525	+	0	0	+	+	+	0	0	0	+	0	+	/	+	0	0	+	+	+	0	0	+	+	+	0	+	0	+		4	+	+
5	r'r	317378	0	+	0	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	+	0	+	+	0	+	+	+	0	+	@	5	+	+
6	r''r	317159	0	0	+	+	+	+	0	0	0	+	0	+	/	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	@	6	+	+
7	rr	320524	0	0	0	+	+	+	0	0	+	+	0	+	/	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	@, HLA+	7	+	+
8	rr	320369	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	+	+	+	+	0	+	+	0	+	0	0	+	@	8	+	+
9	rr	314224	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	HLA+	9	+	+
10	rr	115674	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	0	+s	0	+	HLA+	10	+	+
11	R1R1	320512	+	+	0	0	+	0	0	0	0	+	0	+	/	+	0	+	+	0	+	0	+	+	0	+	0	+s	0	+	HLA+	11	+	+
	Patient Cells																																	
Mode of Reactivity			37°C/Antiglobulin								Antiglobulin											Variable		Cold				Var.					

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment. "/" represents "Not Tested" for new donors.

	Additional Cells		Rh-hr									KELL				DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing	Test Results	
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell #		

Case 2: A 34-year-old first time male donor, A negative blood group with no history of blood transfusion. The O cells and O papanised cells were having positive reaction during reverse grouping and IAT was positive with a negative DAT. Screening cell (I, II, III) panel: +2, +2, 0 and 11 cell panel indicated the presence of anti-Le^b with a wide thermal range indicating that it had both IgM and IgG components. Auto-control at 22^0C and 37^0C were negative and 4^0C was positive. Titre of the antibody at 4^0C was found to be 64, at 22^0C was 32 and at 37^0C after DTT treatment was 8. Secretor status of the donor was found to be non- secretor. On phenotyping, the donor was homozygous for Le^a antigen.



REAGENS Kft.
1155 Budapest,
Wysocki u. 1.
Hungary

REF 41150 5 ml
REF 41180 10 ml

IVD CE 1011

24/02/18 Lot no 721803 exp uj →26/03/18-ig

ReaCell I, II, III

szűrősejtek antitestek kimutatására
red cells for antibody screening

vörösvérsejt szuszpenzió / red blood cell suspension: 0,8±0,1%

Gyártási szám / LOT: **721803**
Lejárati idő / Expiry date: **2018. 03. 26.**
Tárolás / Storage: **2-8°C**

Beküldő kórház / Dispatcher hospital:
Beküldő kód / Dispatcher code:
Minta vételének ideje / Date of sampling:
Beküldő orvos / Dispatcher doctor:
Vizsgálat dátuma / Date of examination:

Beteg neve / Name of patient:
Születési ideje / Date of birth: 170183
TAJ száma / Social insurance identifier:
Vércsoport / Blood group: Rh:
Vizsgálati naplószám / Audit log number:
Vizsgáló labor / Examining laboratory:

	Rh-hr	D	C	E	c	e	C^w	K	k	M	N	S	s	P$_1$	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Di^a	Kp^a	Kp^b	Js^a	Js^b	Lu^a	Lu^b	Eredmények / Results 37°C	22°C
I	$R_1R_1^W$	+	+	o	o	+	+	o	+	o	+	o	+	+	o	+	+	o	+	o	o	o	+	n.t.	n.t.	o	+	+	+
II	R_2R_2	+	o	+	+	o	o	o	+	+	o	+	o	+	o	+	+	+	o	+	o	o	+	n.t.	n.t.	o	+	+	+
III	rr K+	o	o	o	+	+	o	+	+	+	+	o	+	+	+	o	o	+	+	+	o	o	+	n.t.	n.t.	o	+	0	0
																												ss.:	

n.t.: nem tesztelt / not tested

A színezett oszlopok jelzik azokat az antigéneket, amelyek enzimek hatására károsodnak, vagy a reakcióképességük gyengülhet.
The highlighted columns indicate antigens get damaged or diminished in reactivity by enzyme treatment.

Megjegyzés / Notice:

Ortho Clinical Diagnostics

PATIENT NAME: 170183

PATIENT ID:

DATE: TECH:

CONCLUSION:

Lot No. RA071 Exp. Date 2017-12-19 (CCYY MM DD)

Panel A

Reagent Red Blood Cells
Resolve® Panel A
ANTIGRAM® Antigen Profile

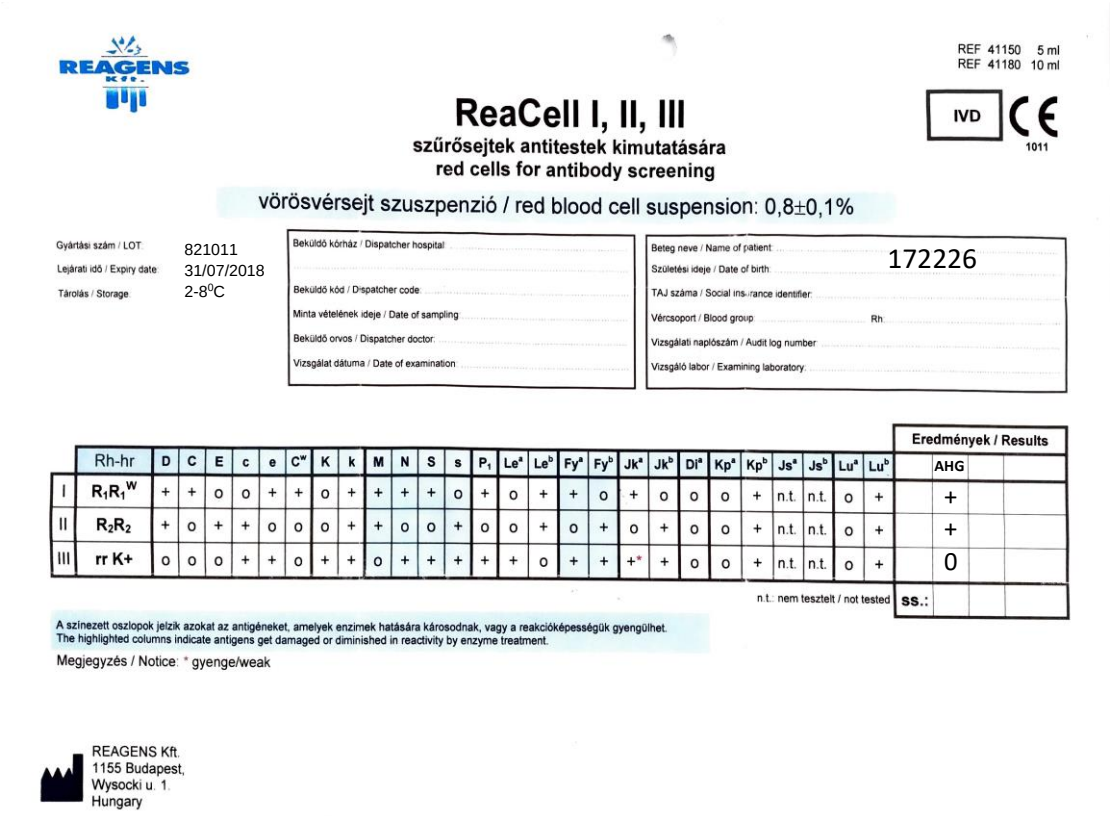
6352007731

			Rh-hr									KELL				DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing		Test Results	
Cell#	Rh-hr	Donor Number	D	C	E	c	e	f	Cw	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N	P1	Lua	Lub		Cell#	AHG	22°C
1	R1wR1	319600	+	+	0	0	+	0	+	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	+	0	0	+		1	+	+
2	R1R1	315054	+	+	0	0	+	0	0	0	+	+	0	+	/	+	+	0	+	+	0	0	0	+	+	+	+	+	+	0		2	0	0
3	R2R2	320515	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	+	+	+	0	+	0	+	0	+	0	+s	+	+		3	0	0
4	Ror	320525	+	0	0	+	+	+	0	0	0	+	0	+	/	+	0	0	+	+	+	0	0	+	+	+	0	+	0	+		4	0	0
5	r'r	317378	0	+	0	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	+	0	+	+	0	+	+	+	0	+	@	5	+	+
6	r''r	317159	0	0	+	+	+	+	0	0	0	+	0	+	/	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	@	6	+	+
7	rr	320524	0	0	0	+	+	+	0	0	+	+	0	+	/	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	@, HLA+	7	+	+
8	rr	320369	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	+	+	+	+	0	+	+	0	+	0	0	+	@	8	0	0
9	rr	314224	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	HLA+	9	+	+
10	rr	115674	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	0	+s	0	+	HLA+	10	+	+
11	R1R1	320512	+	+	0	0	+	0	0	0	0	+	0	+	/	+	0	+	+	0	+	0	+	+	0	+	0	+s	0	+	HLA+	11	+	+
	Patient Cells																																	
Mode of Reactivity			37°C/Antiglobulin									Antiglobulin									Variable		Cold					Var.					

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment. "/" represents "Not Tested" for new donors.

	Additional Cells		Rh-hr									KELL				DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing		Test Results	
Cell#	Rh-hr	Donor Number	D	C	E	c	e	f	Cw	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N	P1	Lua	Lub		Cell#		

Case 3: A 35-year-old first time female AB negative donor, with no previous history of transfusion but has 2 pregnancies with Rh positive offsprings. She had reaction with papanised O cells in reverse grouping, IAT positive, DAT negative and on Screening cells (I, II, III): reactions were +2, +2, 0. Inhouse 6 cell panel revealed the antibody to be anti-D. The IgG component titre was found to be 32.



REAGENS Kft.

REF 41150 5 ml
REF 41180 10 ml

IVD CE 1011

ReaCell I, II, III

szűrősejtek antitestek kimutatására
red cells for antibody screening

vörösvérsejt szuszpenzió / red blood cell suspension: 0,8±0,1%

Gyártási szám / LOT: 821011
Lejárati idő / Expiry date: 31/07/2018
Tárolás / Storage: 2-8°C

Beküldő kórház / Dispatcher hospital:
Beküldő kód / Dispatcher code:
Minta vételének ideje / Date of sampling:
Beküldő orvos / Dispatcher doctor:
Vizsgálat dátuma / Date of examination:

Beteg neve / Name of patient: 172226
Születési ideje / Date of birth:
TAJ száma / Social insurance identifier:
Vércsoport / Blood group: Rh:
Vizsgálati naplószám / Audit log number:
Vizsgáló labor / Examining laboratory:

	Rh-hr	D	C	E	c	e	C^w	K	k	M	N	S	s	P_1	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Di^a	Kp^a	Kp^b	Js^a	Js^b	Lu^a	Lu^b	Eredmények / Results AHG
I	$R_1R_1^W$	+	+	o	o	+	+	o	+	+	+	+	o	+	o	+	+	o	+	o	o	o	+	n.t.	n.t.	o	+	+
II	R_2R_2	+	o	+	+	o	o	o	+	+	o	o	+	o	o	+	o	+	o	+	o	o	+	n.t.	n.t.	o	+	+
III	rr K+	o	o	o	+	+	o	+	+	o	+	+	+	+	+	o	+	+	+*	+	o	o	+	n.t.	n.t.	o	+	0

n.t.: nem tesztelt / not tested

ss.:

A színezett oszlopok jelzik azokat az antigéneket, amelyek enzimek hatására károsodnak, vagy a reakcióképességük gyengülhet.
The highlighted columns indicate antigens get damaged or diminished in reactivity by enzyme treatment.

Megjegyzés / Notice: * gyenge/weak

REAGENS Kft.
1155 Budapest,
Wysocki u. 1.
Hungary

Sl no	Rh-hr	D	C	c	E	e	K	k	M	N	S	s	P1	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Lu^a	Lu^b	Cell No.	AHG
1	R1R1	+	+	0	0	+	0	+	+	+	+	+	+	0	+	0	+	0	+	0	+	1	+
2	R1R1	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	2	+
3	R2R2	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	3	+
4	rr	0	0	+	0	+	+	+	+	+	+	0	+	0	+	+	+	+	+	0	+	4	0
5	rr	0	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	+	0	0	+	5	0
6	rr	0	0	+	0	+	0	+	+	+	+	+	+	+	+	0	+	0	+	0	+	6	0
A/c	rr	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+	0	+	A/c	0

Case 4: A 34-year-old repeat male donor with blood group B positive, with no history of blood transfusion in the past. He had a blood group discrepancy in reverse grouping. 2+ agglutination with O cell at 37^0C and negative DAT. On Screening cells (I, II, III), there was reaction at 37^0C saline phase: 2+, 2+, 0.

Reagent	Anti-A	Anti-B	Anti-A1	Anti-A1	Anti-H	Anti-D (IgM+IgG)	Anti-D (IgM)
Reaction	0	4+	4+	0	2+	3+	3+

Reagent	A1 cells	B cells	O cells	Pap O cells
Reaction	4+	1+	2+	0

Auto-control at 22^0C and 37^0C was negative but saline phase at 4^0C was positive. On inhouse 6 cell panel, the antibody was identified as anti-M which had a wide thermal range. IgM titre was 64 and IgG titre was 16. On phenotyping: homozygous for N antigen.

REAGENS Kft.

REF 41150 5 ml
REF 41180 10 ml

IVD CE 1011

ReaCell I, II, III

szűrősejtek antitestek kimutatására
red cells for antibody screening

vörösvérsejt szuszpenzió / red blood cell suspension: 0,8±0,1%

Gyártási szám / LOT: **721807**
Lejárati idő / Expiry date: **2018. 05. 25.**
Tárolás / Storage: **2-8°C**

Beküldő kórház / Dispatcher hospital:
Beküldő kód / Dispatcher code:
Minta vételének ideje / Date of sampling:
Beküldő orvos / Dispatcher doctor:
Vizsgálat dátuma / Date of examination:

Beteg neve / Name of patient:
Születési ideje / Date of birth: 170272
TAJ száma / Social insurance identifier:
Vércsoport / Blood group: Rh:
Vizsgálati naplószám / Audit log number:
Vizsgáló labor / Examining laboratory:

	Rh-hr	D	C	E	c	e	C^w	K	k	M	N	S	s	P_1	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Di^a	Kp^a	Kp^b	Js^a	Js^b	Lu^a	Lu^b	Eredmények / Results 37°C
I	$R_1R_1^W$	+	+	o	o	+	+	o	+	+	+	+	o	+	o	+	+	o	+	o	o	o	+	n.t.	n.t.	o	+	
II	R_2R_2	+	o	+	+	o	o	o	+	+	o	o	+	o	o	+	o	+	o	+	o	o	+	n.t.	n.t.	o	+	+
III	rr K+	o	o	o	+	+	o	+	+	o	+	+	+	+	+	o	+	+	+*	+	o	o	+	n.t.	n.t.	o	+	0
																												ss.:

n.t.: nem tesztelt / not tested

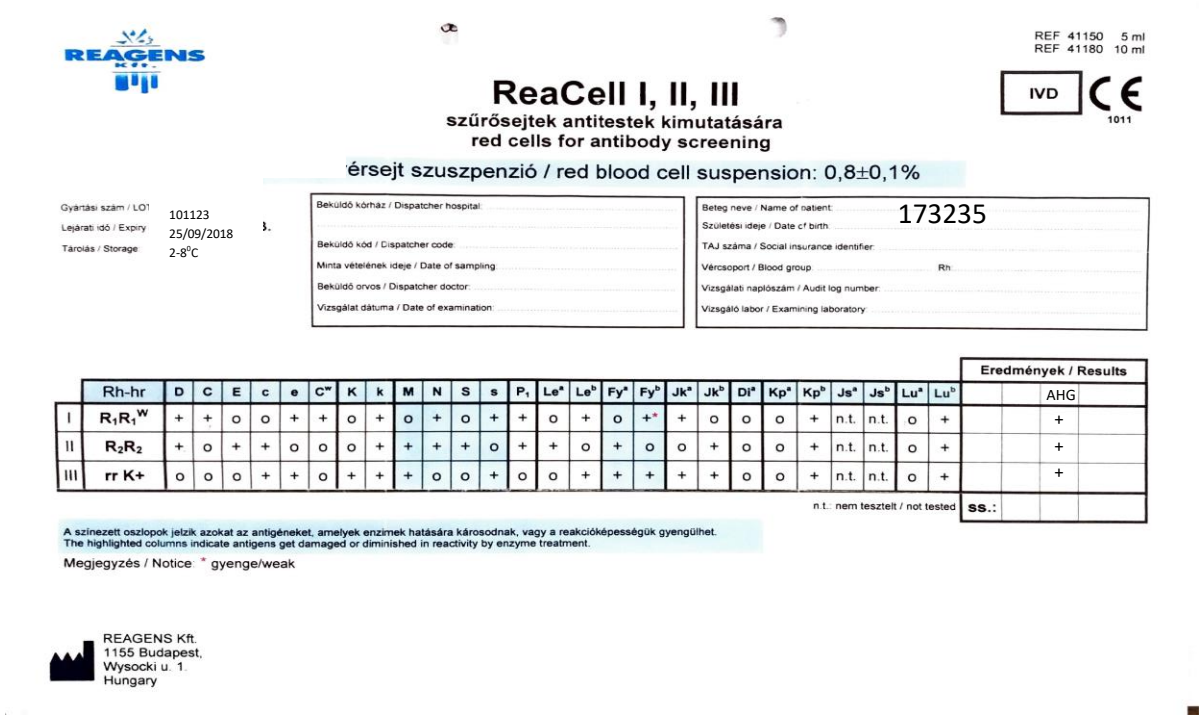
A színezett oszlopok jelzik azokat az antigéneket, amelyek enzimek hatására károsodnak, vagy a reakcióképességük gyengülhet.
The highlighted columns indicate antigens get damaged or diminished in reactivity by enzyme treatment.

Megjegyzés / Notice: * gyenge/weak

REAGENS Kft.
1155 Budapest,
Wysocki u. 1.
Hungary

Sl no	Rh-hr	D	C	c	E	e	K	k	M	N	S	s	P1	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Lu^a	Lu^b	Cell No.	37 C	22 C	4C
1	R1R1	+	+	0	0	+	0	+	+	+	+	+	+	0	+	0	+	0	+	0	+	1	+	+	+
2	R1R1	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	2	0	0	+
3	R2R2	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	3	+	+	+
4	rr	0	0	+	0	+	+	+	+	+	+	0	+	0	+	+	+	+	+	0	+	4	+	+	+
5	rr	0	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	+	0	0	+	5	+	+	+
6	rr	0	0	+	0	+	0	+	+	+	+	+	0	+	+	0	+	0	+	0	+	6	+	+	+
A/c	rr	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+	0	+	A/c	0	0	+

Case 5: A 26-year-old first time male donor, B positive, with no history of transfusion in past. Papanised O cells came positive. He had a positive IAT and negative DAT. 3 cell screening panel gave pan reactivity.



REF 41150 5 ml
REF 41180 10 ml

IVD CE 1011

ReaCell I, II, III
szűrősejtek antitestek kimutatására
red cells for antibody screening

érsejt szuszpenzió / red blood cell suspension: 0,8±0,1%

Gyártási szám / LOT: 101123
Lejárati idő / Expiry: 25/09/2018
Tárolás / Storage: 2-8°C

Beküldő kórház / Dispatcher hospital:
Beküldő kód / Dispatcher code:
Minta vételének ideje / Date of sampling:
Beküldő orvos / Dispatcher doctor:
Vizsgálat dátuma / Date of examination:

Beteg neve / Name of patient: 173235
Születési ideje / Date of birth:
TAJ száma / Social insurance identifier:
Vércsoport / Blood group: Rh:
Vizsgálati naplószám / Audit log number:
Vizsgáló labor / Examining laboratory:

	Rh-hr	D	C	E	c	e	C^w	K	k	M	N	S	s	P$_1$	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Di^a	Kp^a	Kp^b	Js^a	Js^b	Lu^a	Lu^b	Eredmények / Results AHG
I	$R_1R_1^w$	+	+	o	o	+	+	o	+	o	+	o	+	+	o	+	o	+*	+	o	o	o	+	n.t.	n.t.	o	+	+
II	R_2R_2	+	o	+	+	o	o	o	+	+	+	+	o	+	+	o	+	o	o	+	o	o	+	n.t.	n.t.	o	+	+
III	rr K+	o	o	o	+	+	o	+	+	+	o	o	+	o	o	+	+	+	+	+	o	o	+	n.t.	n.t.	o	+	+
																												ss.:

n.t.: nem tesztelt / not tested

A színezett oszlopok jelzik azokat az antigéneket, amelyek enzimek hatására károsodnak, vagy a reakcióképességük gyengülhet.
The highlighted columns indicate antigens get damaged or diminished in reactivity by enzyme treatment.

Megjegyzés / Notice: * gyenge/weak

REAGENS Kft.
1155 Budapest,
Wysocki u. 1.
Hungary

Auto-control at 4^0C, 22^0C and 37^0C: negative and on 11 cell panel, the antibody was identified as anti-Le^{ab}.

Secretor status was assessed and he was a secretor for B and H substance and no Le^a and Le^b substance was found in the saliva. On phenotyping, he was found to be Le (a– b–).

IgM titre: 64 and IgG titre (post DTT treatment): 8.

Ortho Clinical Diagnostics

PATIENT NAME: 172235

PATIENT ID:

DATE: TECH:

CONCLUSION:

Lot No. RA071

Panel A

Reagent Red Blood Cells
Resolve® Panel A
ANTIGRAM® Antigen Profile

635200731

			Rh-hr									KELL				DUFFY		KIDD		Sex Linked	LEWIS		MNS					P	LUTHERAN		Special Antigen Typing		Test Results	
Cell#	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell#	AHG	22C
1	R1wR1	319600	+	+	0	0	+	0	+	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	+	0	0	+		1	+	+
2	R1R1	315054	+	+	0	0	+	0	0	0	+	+	0	+	/	+	+	0	+	+	0	0	0	+	+	+	+	+	+	0		2	0	0
3	R2R2	320515	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	+	+	+	0	+	0	+	0	+	0	+s	+	+		3	+	+
4	Ror	320525	+	0	0	+	+	+	0	0	0	+	0	+	/	+	0	0	+	+	+	0	0	+	+	+	0	+	0	+		4	0	0
5	r'r	317378	0	+	0	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	+	0	+	+	0	+	+	+	0	+	@	5	+	+
6	r''r	317159	0	0	+	+	+	+	0	0	0	+	0	+	/	+	0	+	+	+	0	0	+	0	+	0	+	+	0	+	@	6	+	+
7	rr	320524	0	0	0	+	+	+	0	0	+	+	0	+	/	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	@, HLA+	7	+	+
8	rr	320369	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	+	+	+	+	0	+	+	0	+	0	0	+	@	8	+	+
9	rr	314224	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	HLA+	9	+	+
10	rr	115674	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	0	+s	0	+	HLA+	10	+	+
11	R1R1	320512	+	+	0	0	+	0	0	0	0	+	0	+	/	+	0	+	+	0	+	0	+	+	0	+	0	+s	0	+	HLA+	11	+	+
	Patient Cells																																	
Mode of Reactivity			37°C/Antiglobulin								Antiglobulin											Variable		Cold				Var						

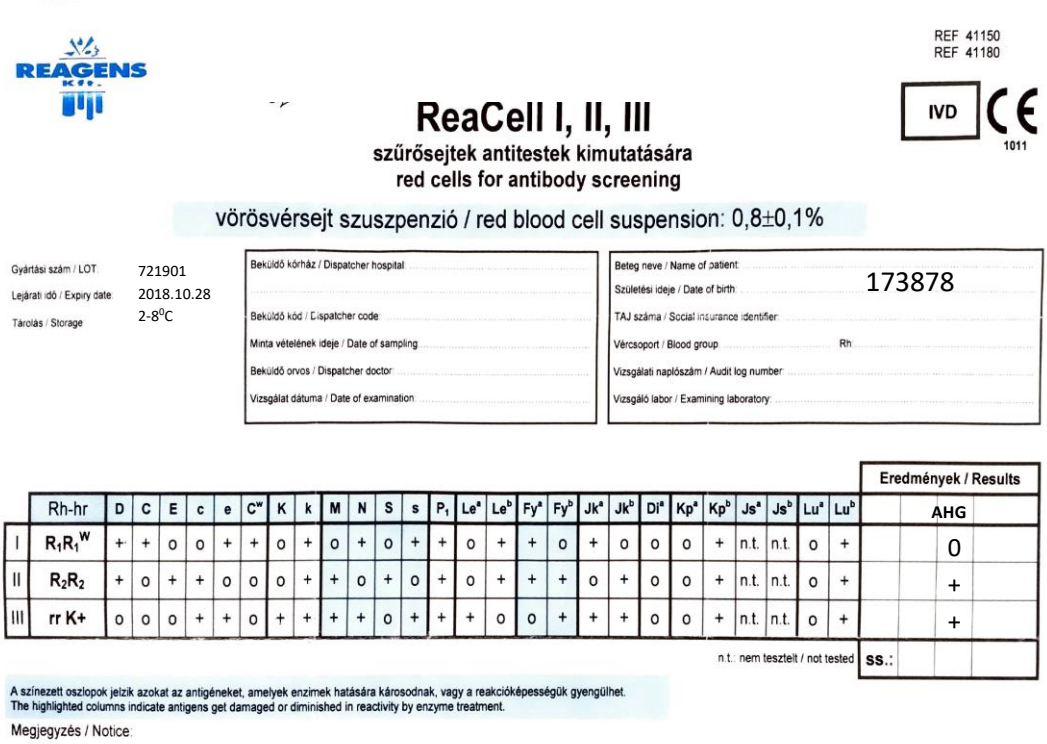
Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment. "/" represents "Not Tested" for new donors.

	Additional Cells		Rh-hr									KELL				DUFFY		KIDD		Sex Linked	LEWIS		MNS					P	LUTHERAN		Special Antigen Typing		Test Results	
Cell#	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell#		

Case 6: A 32-year-old male, repeat donor, O positive had a blood group discrepancy on reverse grouping. DAT negative, IAT positive and auto-control at 22^0C and 37^0C: negative.

Reagent	Anti-A	Anti-B	Anti-A1	Anti-A1	Anti-H	Anti-D (IgM+IgG)	Anti-D (IgM)
Reaction	0	0	0	0	4+	3+	3+

Reagent	A1 cells	B cells	O cells	Pap O cells
Reaction	4+	4+	2+	0



REAGENS Kft.

REF 41150
REF 41180

IVD CE 1011

ReaCell I, II, III
szűrősejtek antitestek kimutatására
red cells for antibody screening

vörösvérsejt szuszpenzió / red blood cell suspension: 0,8±0,1%

Gyártási szám / LOT: 721901
Lejárati idő / Expiry date: 2018.10.28
Tárolás / Storage: 2-8^{0}C

Beküldő kórház / Dispatcher hospital:
Beküldő kód / Dispatcher code:
Minta vételének ideje / Date of sampling:
Beküldő orvos / Dispatcher doctor:
Vizsgálat dátuma / Date of examination:

Beteg neve / Name of patient: 173878
Születési ideje / Date of birth:
TAJ száma / Social insurance identifier:
Vércsoport / Blood group: Rh:
Vizsgálati naplószám / Audit log number:
Vizsgáló labor / Examining laboratory:

	Rh-hr	D	C	E	c	e	C^w	K	k	M	N	S	s	P$_1$	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Di^a	Kp^a	Kp^b	Js^a	Js^b	Lu^a	Lu^b	Eredmények / Results AHG
I	$R_1R_1^W$	+	+	o	o	+	+	o	+	o	+	o	+	+	o	+	+	o	+	o	o	o	+	n.t.	n.t.	o	+	0
II	R_2R_2	+	o	+	+	o	o	o	+	+	o	+	o	+	o	+	+	+	o	+	o	o	+	n.t.	n.t.	o	+	+
III	rr K+	o	o	o	+	+	o	+	+	+	+	o	+	+	+	o	o	+	+	+	o	o	+	n.t.	n.t.	o	+	+

n.t.: nem tesztelt / not tested SS.:

A színezett oszlopok jelzik azokat az antigéneket, amelyek enzimek hatására károsodnak, vagy a reakcióképességük gyengülhet.
The highlighted columns indicate antigens get damaged or diminished in reactivity by enzyme treatment.

Megjegyzés / Notice:

Auto-control at 4^0C, 22^0C and 37^0C: negative. Antibody identified as anti-M and 11 cell panel reactions enhanced at 4^0C. On phenotyping: M(–) N(+). IgM titre: 32 and IgG after DTT treatment: 16.

BIO-RAD

Set ID-DiaPanel: 45161.38.x (Jap[redacted]16.38.xx)
Set ID-DiaPanel P: 45171.38.x (Japan: 4517.38.xx)

LOT 06171.38.x – 06271.38.x (Japan: 0617.[redacted] – 0627.38.xx)
05361.38.x – 05461.38.x (Japan: 0536.38.xx – 0546.38.xx)

ID-DiaPanel
ID-DiaPanel-P

Antigen-Tabelle / Antigen-Table / Table d'antigènes / Tabella antigenica / Tabla de antigenos / Tabela de antigénios
Antikörper-Identifizierung / Antibody identification / Identification d'anticorps / Identificazione anticorpale / Identificación del anticuerpo / Identificação do anticorpos

IVD CE 0123

	Rh-hr	Möglicher Genotyp / Probable Genotype / Genotype probable / Probabile genotipo / Genotipo probable / Genótipo provável	Spender / Donor / Donneur / Donatore / Donante / Dador	D⁻	C	E	c	e	Cʷ	K	k	Kpᵃ	Kpᵇ	Jsᵃ	Jsᵇ	Fyᵃ	Fyᵇ	Jkᵃ	Jkᵇ	Leᵃ	Leᵇ	P₁	M	N	S	s	Luᵃ	Luᵇ	Xgᵃ	♀	♂	Spez. Antigene / Special types / Antigènes part. / Antigeni particolari / Otros Antigenos / Tipos especiais	
1	CCCʷD.ee	$R_1^wR_1$	445591	+	+	0	0	+	+	0	+	0	+	nt	nt	+	0	+	0	0	+	0	+	+	0	+	0	+	+				1
2	CCD.ee	R_1R_1	502657	+	+	0	0	+	0	+	+	0	+	nt	nt	0	+	0	+	+	0	+	+	+	+	0	0	+	+				2
3	ccD.EE	R_2R_2	668540	+	0	+	+	0	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	+	+	0	+	0	+	+				3
4	Ccddee	r′r	281700	0	+	0	+	+	0	0	+	0	+	nt	nt	0	+	+	0	0	0	+	+	0	0	+	+	+	+	+			Co(b+)*	4
5	ccddEe	r″r	230970	0	0	+	+	+	0	0	+	0	+	nt	nt	0	+	+	+	0	+	+	+	0	+	0	0	+	+					5
6	ccddee	rr	608016	0	0	0	+	+	0	+	+	0	+	nt	nt	0	+	+	+	0	+	+	+	0	+	+	+	+	+					6
7	ccddee	rr	018240	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	0	+	0	0	0	0	+	0	+	+	+	+					7
8	ccD.ee	R_or	334083	+	0	0	+	+	0	0	+	0	+	nt	nt	0	0	+	+	0	+	+	+	0	0	+	0	+	0				M1+*	8
9	ccddee	rr	503665	0	0	0	+	+	0	0	+	0	+	nt	nt	+	+	0	+	+	0	0	+	+	+	0	0	+	0					9
10	ccddee	rr	421732	0	0	0	+	+	0	0	+	+	+	nt	nt	0	+	+	0	0	+	+	0	+	0	+	0	+	+					10
11	ccddee	rr	982514	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	0	+	+	0	0	+	+					11
	Patient / Patient / Patient / Paziente / Paciente / Paciente																															Eigenkontrolle / Autocontrol / Autocontrôle / Autocontrollo / Autocontrol / Auto-controlo	

Resultat / Result / Résultat / Risultato / [illegible] / Resultado / Resultado	Bemerkungen / Remarks / Remarques / Note / Observaciones / Observações
0 cell @ 37C	
+	
+	
+	
+	
+	
+	
0	
+	
+	
0	
0	

Anmerkungen siehe rückseitig / Remarks see overleaf / Voir les remarques au verso / Per le note consultare il retro / Ver observaciones en el reverso / Ver observações no verso

Name / Name / Nom / Nome / Nombre / Nome	Blutgruppe + Antigene / Blood group + antigens / Groupe sanguin + antigènes / Gruppo sanguigno + antigeni / Grupo sanguíneo + antigenos / Grupo sanguíneo + antigénios	Interpretation / Interpretation / Interprétation / Interpretazione / Interpretación / Interpretação	Datum / Date / Date / Data / Fecha / Data

B004115 03.18 08.05.2018 / 08:45 V.01 DiaMed GmbH, Pra Rond 23, 1785 Cressier FR, Switzerland, www.bio-rad.com

Case 7: A 29-year-old female, repeat donor, B negative blood group, with a past history of pregnancy (2 years back) and no history of transfusion. She received anti-D prophylaxis after her delivery. The IAT was positive and DAT came negative. Auto-control negative at 4^0C, 22^0C and 37^0C. On papanised O cells reaction enhanced, 3 cell screening panel gave 2+, 0, 0 reaction. On 11 cell panel, antibody identified as anti-C with IgG titre of 8.

REAGENS Kft.

REF 41150 5 ml
REF 41180 10 ml

IVD CE 1011

ReaCell I, II, III

…zűrősejtek antitestek kimutatására
red cells for antibody screening

vörösvérsejt szuszpenzió / red blood cell suspension: 0,8±0,1%

Gyártási szám / LOT: 721901
Lejárati idő / Expiry d: 2019.02.28
Tárolás / Storage: 2-8^{0}C

Beküldő kórház / Dispatcher hospital:
Beküldő kód / Dispatcher code:
Minta vételének ideje / Date of sampling:
Beküldő orvos / Dispatcher doctor:
Vizsgálat dátuma / Date of examination:

Beteg neve / Name of patient: 174954
Születési ideje / Date of birth:
TAJ száma / Social insurance identifier:
Vércsoport / Blood group: Rh:
Vizsgálati naplószám / Audit log number:
Vizsgáló labor / Examining laboratory:

	Rh-hr	D	C	E	c	e	C^w	K	k	M	N	S	s	P_1	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Di^a	Kp^a	Kp^b	Js^a	Js^b	Lu^a	Lu^b	Eredmények / Results AHG
I	$R_1R_1^W$	+	+	o	o	+	+	o	+	o	+	o	+	+	o	+	+	o	+	o	o	o	+	n.t.	n.t.	o	+	+
II	R_2R_2	+	o	+	+	o	o	o	+	+	o	+	o	+	o	+	+	+	o	+	o	o	+	n.t.	n.t.	o	+	0
III	rr K+	o	o	o	+	+	o	+	+	+	+	o	+	+	+	o	o	+	+	+	o	o	+	n.t.	n.t.	o	+	0

n.t.: nem tesztelt / not tested

ss.:

A színezett oszlopok jelzik azokat az antigéneket, amelyek enzimek hatására károsodnak, vagy a reakcióképességük gyengülhet.
The highlighted columns indicate antigens get damaged or diminished in reactivity by enzyme treatment.

Megjegyzés / Notice:

BIO-RAD

Set ID-DiaPanel: 45161.38.x (Jap[illegible]16.38.xx) **LOT** 06171.38.x – 06271.38.x (Japan: 0617.[illegible]– 0627.38.x)
Set ID-DiaPanel P: 45171.38.x (Japan: 4517.38.xx) 05361.38.x – 05461.38.x (Japan: 0536.38.xx – 0546.38.x)

ID-DiaPanel
ID-DiaPanel-P

Antigen-Tabelle / Antigen-Table / Table d'antigènes / Tabella antigenica / Tabla de antígenos / Tabela de antigénios
Antikörper-Identifizierung / Antibody identification / Identification d'anticorps / Identificazione anticorpale / Identificación del anticuerpo / Identificação do anticorpos

IVD CE 0123

	Rh-hr	Möglicher Genotyp / Probable Genotype / Genotype probable / Probabile genotipo / Genotipo probable / Genótipo provável	Spender / Donor / Donneur / Donatore / Donante / Dador	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth.		Xg		Spez. Antigene / Special types / Antigènes part. / Antigeni particolari / Otros Antigenos / Tipos especiais	
				D⁻	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	♀	♂		
1	CCC^{w}D.ee	$R_1{}^wR_1$	445591	+	+	0	0	+	+	0	+	0	+	nt	nt	+	0	+	0	0	+	0	+	+	0	+	0	+	+				1
2	CCD.ee	R_1R_1	502657	+	+	0	0	+	0	+	+	0	+	nt	nt	0	+	0	+	+	0	+	+	+	+	0	0	+	+				2
3	ccD.EE	R_2R_2	668540	+	0	+	+	0	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	+	+	0	+	0	+	+				3
4	Ccddee	r´r	281700	0	+	0	+	+	0	0	+	0	+	nt	nt	0	+	+	0	0	0	+	+	+	0	+	+	+	+	+		Co(b+)*	4
5	ccddEe	r´´r	230970	0	0	+	+	+	0	0	+	0	+	nt	nt	0	+	+	+	0	+	+	+	0	+	0	0	+	+				5
6	ccddee	rr	608016	0	0	0	+	+	0	+	+	0	+	nt	nt	0	+	+	+	0	+	+	+	0	+	+	0	+	+				6
7	ccddee	rr	018240	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	0	+	0	0	0	0	+	0	+	+	+	+				7
8	ccD.ee	R_or	334083	+	0	0	+	+	0	0	+	0	+	nt	nt	0	0	+	+	0	+	+	+	0	0	+	0	+	0			M1+*	8
9	ccddee	rr	503665	0	0	0	+	+	0	0	+	0	+	nt	nt	+	+	0	+	+	0	0	+	+	+	0	0	+	0				9
10	ccddee	rr	421732	0	0	0	+	+	0	0	+	+	+	nt	nt	0	+	+	0	0	+	+	0	+	0	+	0	+	+				10
11	ccddee	rr	982514	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	0	+	+	0	0	+	+				11
	Patient / Patient / Patient / Paziente / Paciente / Paciente																														Eigenkontrolle / Autocontrol / Autocontrôle / Autocontrollo / Autocontrol / Auto-controlo	

Resultat / Result / Résultat / Risultato / Resultado	Bemerkungen / Remarks / Remarques / Note / Observaciones / Observações
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Anmerkungen siehe rückseitig / Remarks see overleaf / Voir les remarques au verso / Per le note consultare il retro / Ver observaciones en el reverso / Ver observações no verso

Name / Name / Nom / Nome / Nombre / Nome	Blutgruppe + Antigene / Blood group + antigens / Groupe sanguin + antigènes / Gruppo sanguigno + antigeni / Grupo sanguíneo + antígenos / Grupo sanguíneo + antigénios	Interpretation / Interpretation / Interprétation / Interpretazione / Interpretación / Interpretação	Datum / Date / Date / Data / Fecha / Data

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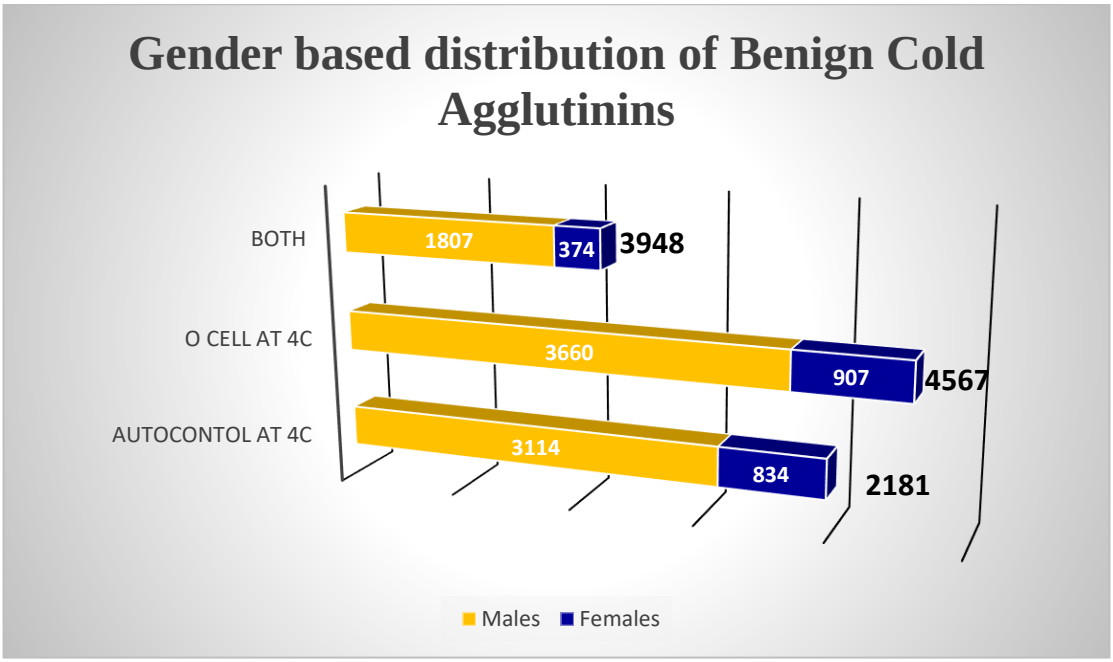
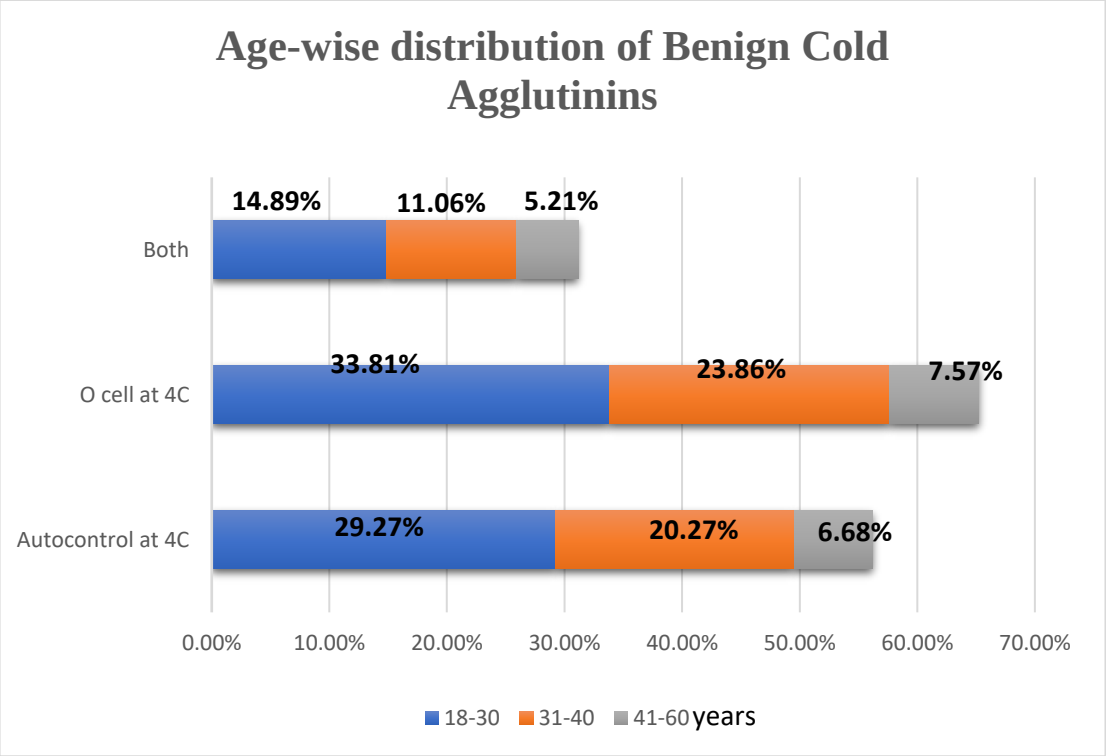
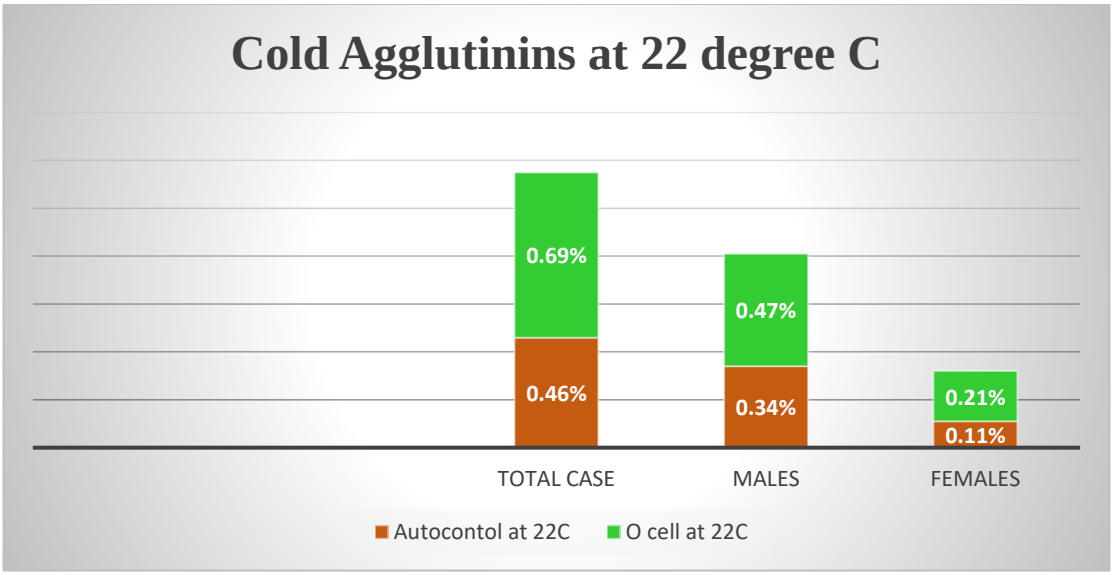
Benign Cold Agglutinins

Cold autoantibodies are often detected in healthy individuals, with the majority being benign and IgM in nature. These antibodies react best at 4^0C. The prevalence of benign cold agglutinins (BCA) at 4^0C for auto-control as well as with pooled O cells were 56.40% and 65.24% respectively and 31.16% of donors showed reaction in both auto-control and pooled O cells. The chi-square test using R & C table in OpenEpi (online software) [61] was used to find out the significance among the gender and age-wise distribution of occurrence of BCA. It was found that there was statistically significant difference observed in gender distribution (*p* value <0.001) and age-wise distribution (*p* value <0.001) stating that the male blood donors had more prevalence of BCA than female blood donor and also the age group of 18-30 years had more BCAs.

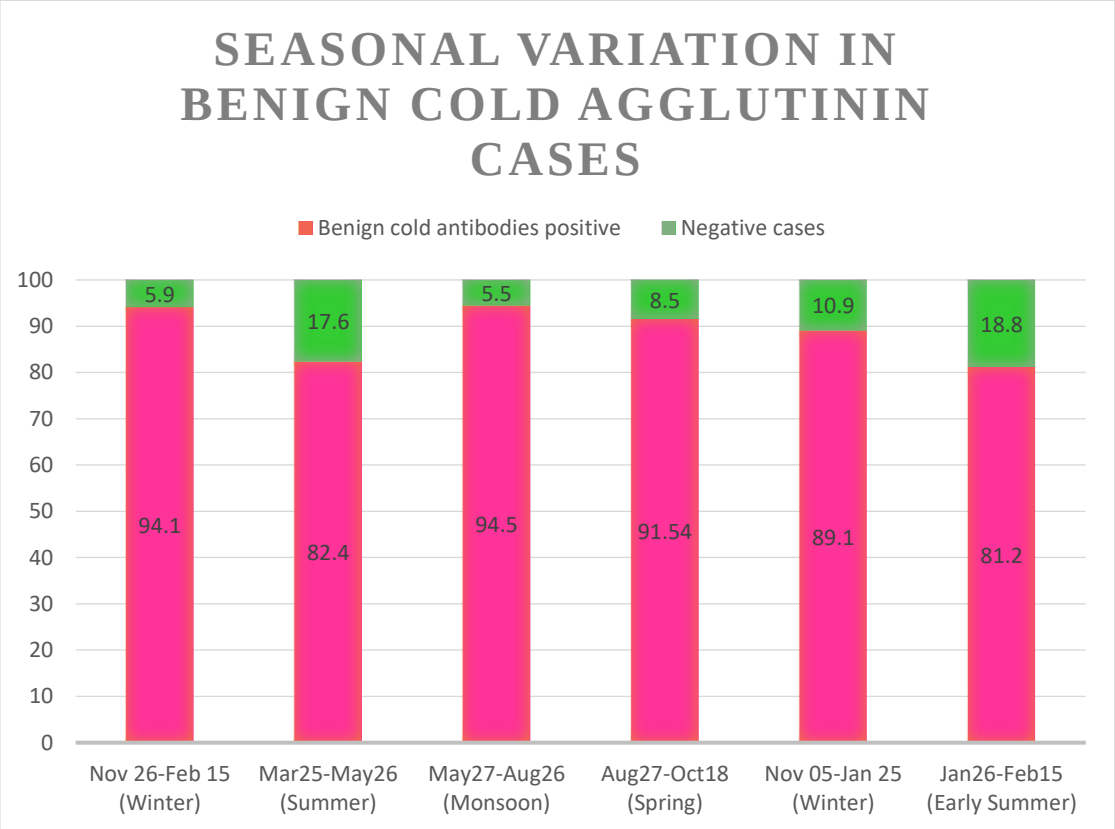




The prevalence of IgM antibodies reacting at 22^0C were 0.46% for auto-control and 0.69% for pooled O cells respectively. Here also, the prevalence was more with males compared to females.



Another interesting finding observed in the prevalence of BCA was the seasonal variations observed in the frequency of cases. Kerala is located close to the equator and hence experiences a tropical climate. Trivandrum, the capital city of Kerala, is a coastal city with the temperatures stay fairly consistent throughout the year. The overall average daily temperature doesn't drop below 27°C or rise above 29°C in the course of the year. The winter season in Kerala is between the end of November until the middle of February and the temperature aren't very different from those experienced in the summer months. Kerala has two rainy seasons: Southwest monsoon (June – August) and Northeast monsoon (October – November). Summer season is between mid-February till end of May and the spring or harvest season is during end of August till end of September. In the present study, the BCA cases were more during monsoon seasons and winter season. The cases during summer season were relatively lower when compared to the cold weather.



During the month of August to September 2018, Kerala was hit by heavy rainfall and floods and hence the cases of BCA were more during this time interval which was otherwise the period of spring season in Kerala with a warm weather.

DISCUSSION

Various worldwide literature calls out the importance of donor unexpected antibody screening and also a topic of debate whether to make this mandatory for the recipient safety. The guidelines for the screening of donor blood to identify iso-immune erythrocyte antibodies have been laid down in National Blood Policy (62), 2007 (National Aids Control Organization/NACO, Ministry of Health & Family Welfare). DGHS, Ministry of Health & Family Welfare (Government of India) (63) also warrants screening of donated blood for irregular erythrocyte antibodies.

Red cell allo-immunisation rates

The incidence of RBC alloimmunization depends largely upon the demography of the population studied (64). Few international studies states that the prevalence of red cell allo- and autoantibodies has been reported in several study populations including hospital based patients, transfusion dependent patients with chronic haematological disorders, pregnant females and blood donors, and the incidence of alloantibodies detected worldwide is 0.2%-0.9% in healthy blood donors, 2%-9% in patients with a history of blood transfusion, 9%-30% among chronic transfusion dependant patients, 0.5%-1.9% among antenatal women and 0.5%-1% of red cell autoantibodies among transfused patients (3, 65-71).

Global literature states that the reported incidence of erythrocyte allo-antibodies in the donor population varies from 0.32 to 2.4% (3,4,6,8-14). REDS III study (Recipient Epidemiology and Donor Evaluation Study) gives inference of a positive antibody screen noted overall in 0.5% of donations and 0.77% of blood donors at the four centres in US included in the study over the 4.5-year study period, utilizing methodologies ranging from solid phase to gel card to tube testing (34). Comparative evaluation of

allo-immunization rates is difficult as different studies have used different serological techniques. In the present study we followed tube technique which is less sensitive than column agglutination technology/solid phase haematological procedures. From the current study on healthy voluntary donors, the prevalence of allo-antibodies is 0.1%. This may be because our population of blood donors are 100% voluntary non-remunerated healthy people who are highly motivated and having good awareness on blood donation. Moreover, we follow a stringent selection of blood donors attending our camp and inhouse blood donations.

Nature of allo-antibodies

A study on the Shaoguan area describes that frequency of irregular antibodies in female was higher than that in male and Rh blood group antibodies (47.6%) such as anti-D, anti-E, and anti-c were common (65). Another US study on male military veterans, the prevalence of red cell antibodies was in the increasing order of K, E, D, Le^a, Fy^a, c, C, P_1, Jk^a, and Le^b (72). Another study from Minnesota, the most frequent allo-antibodies were E, Le^a, K, D, Le^b, M, P_1, Fy^a, C & c in the order (67). A study on frequency of allo-immunisation in Kuwaiti population describes that five most frequently identified alloantibodies were anti-D, anti-E, anti-K, anti-Le^a & anti-Le^b in the descending order (25). Malaysian study on prevalence and specificities of red cell alloantibodies among blood recipients shows that anti-E antibody comprised the most common alloantibody followed by the anti-Le^a antibodies & the anti-M antibody (69). A study done in Southern Thai population showed following antibodies frequency wise as most common – anti-Mia, anti-E, anti-Lea, anti-c and anti-Le^b (70).

Several Indian studies mostly in Northern population are available in the literature. 0.9% prevalence of irregular red cell antibodies was noted among healthy donors in Delhi population (73). The most common alloantibodies were of MNS system (39.13%) followed by anti-D (13%) and anti-Lewis (10.8%). Another similar study involving blood donors in northern India showed the prevalence of 0.05% alloantibodies was observed (64). A tertiary care centre in northern India reported positive screening cells and pooled O cells in 0.27% of donors, 66.08% of these donors had autoantibodies, 1 had auto-antibodies with underlying alloantibody anti-Jka (0.001%), and 0.09% had alloantibodies alone in their plasma. Anti-M was the most common antibody (18.94%) identified, followed by anti-D (9.25%). Another study from State of Odisha, India done on patient population shows that weak/missing antibody, weak antigen expression, rouleaux, cold autoantibodies, cold alloantibodies, Bombay phenotype with the frequency of 13.51%, 2.70%, 2.70%, 54.06%, 8.11%, 18.92% respectively (74). In Indian literature on red cell allo-antibodies, males have higher incidence when compared to females (mostly with a history of pregnancy). In Indian scenario, female blood donations are fewer when compared to the male blood donations and hence this may be a reason that the female population with positive red cell alloimmunisation may be a tip of iceberg phenomenon.

In our study, the prevalence of irregular antibodies was much lower than other national and international studies (0.01%). Moreover, the most common alloimmunisation identified were anti-M and anti-Lewis antibodies (0.03% each), followed by Rh antibodies (anti-C and anti-D, 0.01% each) and anti-IH (0.01%) which is comparable with other Indian studies. Here, the males were having higher incidence of red cell

alloimmunisation and all of them were devoid of a prior sensitisation via blood transfusion indicating that all the 5 cases were naturally occurring allo-antibodies. Whereas females with irregular antibody belong to Rh antibodies (anti-D and anti-C) and results from a previous history of pregnancy.

First time donor Vs Repeat donors

Myhre et al reported that an average of 0.14% of the total units of blood yearly contain unexpected red cell antibodies and this group of donors includes mainly first time donors (84%), but contains in addition a few repeat donors who had not given blood since their screening program began or who had been recently sensitized (71). Since 25% of the total donor population in their study composed of first-time donors, the percentage of antibodies found in first time donors is 0.56%. Similar finding was observed in our study with most of the irregular antibodies were seen among the first-time donors and the repeat donors who came positive were recently sensitized.

Gender distribution of allo-antibodies

In our study, we found that alloimmunisation was common among males than females, but males had naturally occurring antibodies and females had allo-antibodies as a result of past pregnancy. Male to female ratio of alloantibodies was found to be 8.25:1 in Garg N et al study (73) and 4.84:1 in Makroo et al study (33). These studies also pointed out the female allo-immunisation resulted out from past pregnancy whereas males had mostly naturally occurring allo-antibodies followed by past history of blood transfusion.

DAT positivity among study population

In present study, there was no case of auto-immunisation or DAT positivity in total 7000 healthy blood donors studied. Issitt and Anstee (51) reported that, of blood donors with a positive DAT and IgG coating the RBCs, 5–10% will develop AIHA, 20–25% will become DAT negative over time, and 60–70% will remain DAT positive but hematologically normal. The incidence of strongly positive DATs in healthy individuals was 1:3300 in a Canadian study (75). Another French study in 1980, the incidence was found to be 1: 13000 (76). Incidence of a positive DAT amongst the healthy blood donors was 0.04% in an Indian study done in northern Indian population by Tiwari et al (29). Makroo et al (33) states that in their study, the prevalence of DAT positivity among healthy donors was 0.18% and in Kaur et al study, it was 0.05% (64).

Benign cold agglutinin distribution

Cold autoantibodies are often detected in healthy individuals, with the majority being benign which react more strongly at 0–4^{0}C than at warmer temperatures. Pathological cold auto-antibodies are characterized by wide thermal amplitude and/or high antibody titres (56). Joshi SR et al states that cold auto-antibodies observed in their study were found among blood donors with no apparent health problems and may be due to an exposure to infectious agents as antibody stimulant in past was open as the population in the city of Surat was exposed to vector-borne infections such as malaria, gastroenteritis, and infective hepatitis during the contemporary period of this study (55). In the current study, we found prevalence of benign cold agglutinins at 4^{0}C for auto-control as well as with pooled O cells were 56.40% and 65.24% respectively and 31.16% of donors showed reaction in

both auto-control and O cells. Since Kerala is a tropical country with near perennial rainfall and vector borne diseases like dengue, etc. as well as frequent viral upper respiratory infections, the occurrence of cold agglutinins may be associated with the above. Cold agglutinins active at 22^0C are of very low prevalence (<1%) from the current study.

CONCLUSION

Out of 7000 healthy voluntary blood donor population, the prevalence of irregular red cell antibodies was found to be 1in 1000. Male blood donations were more when compared to female and blood donors among 18-30 years of age were greater in number in the study population. There were no cases of DAT positivity encountered in this study. Male: Female ratio is 2.5:1 and males had naturally occurring allo-antibodies whereas females had a previous history of pregnancy. Anti-M and anti-Lewis antibodies were commonest allo-antibodies followed by anti-Rh (D and C) antibodies and anti-IH in this current study. Benign cold agglutinins were found predominately in the younger male population with a significant seasonal variation noted in the prevalence of these antibodies.

LIMITATIONS OF THE STUDY

The study concentrated only on voluntary healthy blood donors and hence do not represent the general South Kerala population. The efficient pre-donation counselling and medical examination filtered out people suffering from major clinical conditions especially autoimmune diseases.

Conventional spin tube method was used in the current study which is inferior technology when compared with gel card method. Gel card technique is well known for its simplicity, stability of results, dispensation of controls, absence of wash phase with comparable sensitivity and specificity as well as appropriate for antibody screening and identification.

SUMMARY

The aim of this study was to determine the prevalence of irregular red cell antibodies in healthy voluntary donors attending our camp and inhouse blood donations. Institute policy is 100% voluntary blood donations and the donor pool consisted mainly of comprehensive, altruistic and motivated people. Since the literature states a very low prevalence of irregular red cell antibody positive cases among healthy blood donors, a sample size of 7000 was adapted after consulting with the institute statistician. The study was conducted over a period of 1 year and 3 months.

The prevalence of irregular red cell antibodies was found to be 1 in 1000. There were no DAT positive cases obtained during the study period. The seven cases reported were 5 males with no past history of blood transfusion and 2 females (Rh negative) with a history of past pregnancy. Anti-M and anti-Lewis antibodies were commonest followed by anti-Rh (C and D) and anti-IH antibodies.

Another interesting finding was the prevalence of benign cold agglutinins at 4^0C (auto-control as well as with pooled O cells) and was found to be were 56.40% and 65.24% respectively and 31.16% of donors showed reaction in both auto-control and pooled O cells. These BCA positive cases were seen more during winter season and rainy seasons when compared to hot climatic conditions.

The importance of antibody screening is highlighted through this study because all antibodies identified are notorious to cause immune hemolytic transfusion reactions in the recipients. Moreover, the DGHS guidelines states that the antibody screening is mandatory alongside the routine blood grouping and Rh typing of blood donors.

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ANNEXURE

Technical Advisory Committee (Clinical Studies)

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY
THIRUVANANTHAPURAM – 695011, INDIA

TAC Registration No: SCT-/S/2017/613 **Date: 12.07.2017**

Project title: Prevalence of unexpected red cell antibodies in healthy blood donor population in a Tertiary Care centre in South Kerala

Principal Investigator
Dr. Gayathri A M., Resident, Department of Transfusion Medicine, SCTIMST Degree: MBBS
Co-Principal Investigator(s)
Dr. Debasish Gupta, Professor, Department of Transfusion Medicine, SCTIMST Degree: MBBS, MD Transfusion Medicine

Members who participated in the TAC meeting on 28/06/2017

Dr. Rupa Sreedhar (Chairperson)
Dr. Mathew Abraham
Dr. Sylaja P.N
Dr. Syam. K
Dr. Bijulal S
Dr. Varghese T. Panicker
Dr. Jayadevan E.R
Dr. K. Shivakumar (Member Secretary)

Dr. P.N. Sylaja, Dr. Jayadevan E.R and Dr. Syam stayed away from the proceedings when the project in which they are involved as investigator were discussed (#607, 611,609,617,628).

Risk Classification of the project (Minimum/ Moderate/ High): Minimum

Requirement of DSMB: No

Recommended members of DSMB: Not applicable

Recommendations of TAC:
Recommended for consideration of IEC in the light of the responses received from the investigator

The PI may note that there can be no additions / alterations in the documents approved by TAC when they are submitted to the IEC.

Signature of the Member Secretary, TAC (Clinical Studies)

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
तिरुवनन्तपुरम - ६९५०११, केरल, इंडिया
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM
Thiruvananthapuram - 695 011, Kerala, India
(An Institute of National Importance under Govt. of India)
Grams : Chitramet, Phone : +91-471-2443152, Fax : +91-471-2550728 / 2446433, E-mail : sct@sctimst.ac.in, Website : www.sctimst.ac.in

Institutional Ethics Committee
(IEC Regn No. ECR/189/Inst/KL/2013)

SCT/IEC/1075/AUGUST-2017

07.10.2017

Dr. Gayathri AM
Resident
Department of Transfusion Medicine
SCTIMST, Thiruvananthapuram

Dear Dr. Gayathri,

The Institutional Ethics Committee reviewed and discussed your application to conduct the study entitled "PREVALENCE OF UNEXPECTED RED CELL ANTIBODIES IN HEALTHY BLOOD DONOR POPULATION IN A TERTIARY CARE CENTRE IN SOUTH KERALA (IEC/1075)" on 19th August, 2017.

The following documents were reviewed:

Original submission

1. *Covering letter addressed to the Chairman, IEC, SCTIMST dated 14.07.2017 with check list*
2. *TAC Approval Letter*
3. *IEC Application Form*
4. *Project Proposal*
5. *Proforma*
6. *Declaration Form*
7. *CV of Principal Investigator and Co-Principal Investigator*

Revised submission

1. *Covering letter addressed to the Chairman, IEC, SCTIMST dated 20.09.2017 with check list*
2. *Copy of IEC Recommendation Letter dated 21.08.2017*
3. *TAC Approval Letter*
4. *IEC Application Form*
5. *Project Proposal*
6. *Proforma*
7. *Donor Information Sheet in English and Malayalam*
8. *Informed Consent in English and Malayalam*
9. *Blood Donor Questionnaire and Blood Informed Consent Form in English and Malayalam*
10. *CDC Guidelines for Blood Banks*
11. *CV of Principal Investigator and Co-Principal Investigator*

The following members of the Ethics Committee were present at the meeting held on 19th August, 2017 at G. Parthasarathi Board Room, AMCHSS, SCTIMST

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Dr. R V G Menon	M Tech, PhD	Male	Lay Person (Chairman)	No
2.	Dr.Lekha Pandit	MD.DM Neurology, PhD (Bioscience)	Female	Clinician	No
3.	Dr. Kala Kesavan. P	MBBS, MD	Female	Basic Medical Scientist	No
4.	Dr. Rema M. N	MD	Female	Basic Medical Scientist	No
5.	Dr. S S Giri Sankar	LL.M. Ph.D.	Male	Legal Expert	No
6.	Dr. Aneesh V Pillai	BA. LLB (Hons.), LLM, Ph. D, SET (Law)	Male	Legal Expert	No
7.	Mr. Satheesh Chandran	MSW, PGDPM	Male	Lay person/ NGO/ Social Scientist	No
8.	Smt. Sathi Nair	MA (English Literature)	Female	Lay Person	No
9.	Dr. P. Manickam	BSMS, MSc (Epid).,PhD	Male	Health Science Expert/ Social Scientist	No
10.	Dr. Christina George	MD Psychiatry	Female	Clinician	No
11.	Dr. V. Raman Kutty	M D, M Phil, M P H	Male	Health Sciences Expert/Clinician	Yes
12.	Dr. K R S Krishnan	M.E., Ph.D.	Male	Medical Technology	Yes
13.	Dr. Harikrishna Varma PR	Ph.D(Materials Science)	Male	Medical Technology	Yes
14	Dr. Harikrishnan S	MD, DM (Cardiology) DNB (Cardiology)	Male	Clinician	Yes
15.	Dr. Mala Ramanathan	PhD	Female	Social Scientist (Member Secretary)	Yes

IEC Decision

The IEC approved the conduct of the study in the present form.

Remarks:

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,

Mala Ramanathan
Member Secretary, IEC

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM

DONOR INFORMATION SHEET

Title of the study: To study the prevalence of unexpected red cell antibodies in a healthy donor population in a tertiary care centre in South Kerala

Name of Principal Investigator: Dr.Gayathri.A.M.

1. What is the study?
   To study the prevalence of unexpected red cell antibodies in a healthy donor population in a tertiary care centre in South Kerala

2. Who are the participants?
   All voluntary blood donors coming for blood donation at SCTIMST blood bank which includes both in house as well as camp donations during a period of one year

3. What are Unexpected Red cell antibodies?
   Antigens are toxins or other foreign substance which induces an immune response in the body, especially the production of antibodies. Antibodies are blood protein produced in response to and counteracting a specific antigen. They combine chemically with substances which the body recognizes as alien, such as bacteria, viruses, and foreign substances in the blood. Antibodies are of two types namely auto-antibodies (against self-antigens) and allo-antibodies (against foreign antigens). Our ABO blood grouping system is formulated based on the antigens present on RBC surface and antibodies present in the plasma. A group individuals have A antigens on RBC surface and anti-B (antibody) in their plasma. Likewise, B group has B antigen and anti-A, AB group have A & B antigens on cell surface and no antibodies in plasma. O group individuals has no antigens on RBC surface whereas both anti-A & anti-B in their plasma. Presence of Rh antigen determines whether an individual is positive or negative group. Presence of any other antibodies other than the normal described are called "unexpected" red cell antibodies.

4. How the estimation of these antibodies helps you?
   Under Drug and Cosmetic Act 1940, it is mandatory to perform antibody screening along with routine ABO & Rh grouping and transfusion transmitted infection screening. Usually the alloantibodies occur in an individual with a previous history of transfusion, pregnancy, needle sharing, certain infections

and idiopathic (unknown reason). Estimation of allo-antibodies helps these individuals in helping them for finding antigen negative blood groups for their future blood requirements.

Auto-antibodies are found in donors with any autoimmune conditions or other disease conditions. Estimation of these antibodies may aid in early diagnosis of the above mentioned conditions. Also the prevalence of these antibodies in the target population can be evaluated from this study. An added point is that these antibodies may not cause any harm to the donor itself.

5. What is the procedure being done?

   An addition 2ml of blood sample will be collected from the tubings of the blood bag along with other pilot tubes taken for grouping, compatibility testing and infection screening. No other additional pricking or any other invasive procedure is needed. From this additional sample antibody screening (Direct Antiglobulin Test & Indirect Antiglobulin Test) is done.

6. What is the benefit for you from the study?

   As I mentioned above, for alloantigen estimation will help you in finding antigen negative blood in future blood requirements & autoantibody estimation may help you in early diagnosis of certain unidentified disease conditions. There is no monetary burden from your side. The individuals with positive auto-antibodies will be further evaluated by a physician.

7. Any risk related to this study?

   There are no inherent risks envisaged in this study.

8. How does this study help the common public?

   This study is performed on voluntary healthy population and detection of unexpected antibodies may help the common public in obtaining appropriate blood units if any future blood requirements arise and also in identifying occult disease conditions in the early stage itself. This study also helps in aiding best transfusion practices to be followed in the institution since there is scarce data available on prevalence and type of irregular antibodies in Indian donor population

9. Can you withdraw from this study after it starts?

Your participation in this study is entirely voluntary and you are also free to withdraw from the data collection part of the study at any point of time, without giving any reason, without your medical care or legal rights being affected

10. Will your personal details be kept confidential?

    This research work if approved will be published as Thesis or in any Transfusion Medicine Journal. But none of the personal data will be mentioned

anywhere in it. However, your medical notes may be reviewed by the ethical committee members and regulatory authorities associated with the study, without your additional permission.

*For any queries, please contact myself Dr. Gayathri.A.M. (mob: 9446208632),or Dr. Debasish Gupta (tel: 04712524177)
email: gayathri@sctimst.ac.in /dgupta@sctimst.ac.in

Name of the Principal Investigator (PI): Dr. Gayathri.A.M.

Address and Contact Details: Junior Resident, Department of Transfusion Medicine, SCTIMST, Trivandrum-695011, Contact-04712524636/9446208632

Signature of the PI

Date:

For any clarifications regarding the study's ethics clearance you may contact Dr. Mala Ramanathan (Member Secretary of the Ethics Committee- SCTIMST). The phone number is 0471-2524234 and the email id is iec.mem.sec@sctimst.ac.in.

INFORMED CONSENT

STUDY TITLE: Prevalence of Unexpected Red Cell Antibodies in Healthy Donor Population in a Tertiary Care Centre in South Kerala.

STUDY NUMBER:

PARTICIPANT'S INITIAL:

DATE OF BIRTH:

DATE:

I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose. I agree to take part in the above study.

Signature (or Thumb impression) of the participant/ Signature of the Investigator

Legally Acceptable Representative: Date:

Date: Study Investigator's Name:

Signatory's Name:

ശ്രീ ചിത്ര തിരുന്നാൾ ഇൻസ്റ്റിട്ട്യൂട്ട് ഫോർ സയൻസ് ആന്റ് ടെക്നോളജി

രക്തദാതാവിനുള്ള കാര്യ വിവരണ പത്രിക

പഠനത്തിന്റെ തലക്കെട്ട് : ദക്ഷിണ കേരളത്തിലെ ഒരു ത്രിതല ആശുപത്രിയിലെത്തുന്ന രക്തദാതാക്കളുടെ ചുമന്ന രക്താണുക്കളിലുള്ള അപ്രതീക്ഷിത ആൻറിബോഡികളുടെ ആധികൃത്തെക്കുറിച്ചുള്ള പഠനം

പ്രധാന നിരീക്ഷകയുടെ പേര്: ഡോ. ഗായത്രി .എ എം.

1. എന്താണ് ഈ പഠനം?
ദക്ഷിണ കേരളത്തിലെ ഒരു ത്രിതല ആശുപത്രിയിലെത്തുന്ന രക്തദാതാക്കളുടെ ചുമന്ന രക്താണുക്കളിലുള്ള അപ്രതീക്ഷിത ആൻറിബോഡികളുടെ ആധികൃത്തെക്കുറിച്ചുള്ള പഠനം

2. ആരൊക്കെയാണ് പങ്കെടുക്കുന്നവർ ?
ശ്രീ ചിത്ര ആശുപത്രിയിലെ രക്ത ബാങ്കിലെത്തുന്നതോ സംഘടിത ക്യാമ്പിലോ രക്തദാനത്തിനായി എത്തുന്ന എല്ലാ സന്നദ്ധ രക്തദാതാക്കൾ ഈ പഠനത്തിന്റെ ഭാഗമാണ്. ഒരു വർഷത്തെ ദൈർഘ്യമാണ് ഈ പഠനത്തിനുള്ളത്.
3. എന്താണ് അപ്രതീക്ഷിത ആൻറിബോഡികൾ?
ശരീരത്തിലെ പ്രതിരോധ ശക്തിയെ സ്വാധീനിക്കാൻ കഴിവുള്ള ഒരു അപ്രസ്തുത വസ്തു/വിഷപദാർത്ഥത്തെ ആന്റിജൻ എന്നു വിളിക്കുന്നു. അനന്തരഫലമായി ശരീരത്തിന്റെ പ്രതിരോധ ശക്തിയുടെ സഹായത്താൽ ഉത്പാദിപ്പിക്കപ്പെടുന്ന കണികയെ ആന്റിബോഡിയെന്നു വിളിക്കുന്നു. ആന്റിബോഡികൾ അപ്രസ്തുത ആന്റിജനുമായ് മാത്രമേ സാധാരണ ഗതിയിൽ രാസപ്രവർത്തനത്തിൽ ഏർപ്പെടുമുള്ളു. ഇവ രണ്ടുതരമുണ്ട്: ആട്ടോ ആന്റിബോഡികളും (ശരീരത്തിലെ തന്നെ ആൻറിജനെതിരെ)അല്ലോ ആൻറിബോഡികളും (ശരീരത്തിനു അന്യമായ ആന്റിജനെതിരെ).
എ, ബി, ഒ രക്ത ഗ്രൂപ്പുകൾ നിലവിൽ വന്നത് വിവിധതരം ആൻറിജനും ആൻറിബോഡികളും ചുവന്ന രക്താണുക്കളിലും പ്ലാസ്മ(രക്തത്തിലുള്ള ദ്രവം) യിലുമുള്ളത് കാരണമാണ്. എ ഗ്രൂപ്പുകാരുടെ ചുമന്ന രക്താണുക്കളിൽ എ ആന്റിജനും പ്ലാസ്മയിൽ ബി ആൻറിബോഡിയുമുണ്ട്. തതുല്യമായ് ബി ഗ്രൂപ്പുകൾക്ക് ബി ആൻറിജൻ രക്താണവിലും പ്ലാസ്മയിൽ എ ആന്റിബോഡിയും കാണപ്പെടുന്നു. ഒ ഗ്രൂപ്പുകാർ ചുമന്ന രക്താണുക്കളിൽ എ, ബി ആൻറിജനുകൾ ഇല്ല, പക്ഷേ പ്ലാസ്മയിൽ എ ,ബി ആൻറിബോഡികൾ ഉണ്ട്. എ ബി ഗ്രൂപ്പുകാരിൽ എ ബി ആർറിജനുകൾ രക്താണുക്കളിൽ കാണപ്പെടുന്നു പക്ഷേ പ്ലാസ്മയിൽ ഏ, ബി ആൻറിബോഡികൾ ഇല്ല. കൂടാതെ ഒരു വ്യക്തി ഗ്രൂപ്പ് പോസിറ്റീവ് / നെഗറ്റീവ് എന്ന് തീരുമാനിക്കുന്നത് അവരിലെ ചുവന്ന രക്താണുക്കളിലെ ആർ ഹെച്ച് ആൻറിജന്റെ സാന്നിദ്ധ്യം ഉള്ളതോ/ ഇല്ലാത്തതോ മൂലമാണ് .
ഇവയല്ലാതെ മറേറതൊരു ആൻറിബോഡി രക്താണുക്കളിൽ കാണപ്പെട്ടാൽ അവയെ അപ്രതീക്ഷിത ആൻറിബോഡിയെന്നു വിളിക്കുന്നു.

4. ഈ ആൻറിബോഡികളുടെ സാന്നിദ്ധ്യത്തെ കണ്ടെത്തുന്നതു നിങ്ങളെ എങ്ങനെ സഹായിക്കുന്നു ?
ഡ്രഗ്സ് ആന്റ് കോസ്മെറ്റിക്സ് ആക്റ്റ്1945 പ്രകാരം എ, ബി, ഒ ഗ്രൂപ്പു നിർണ്ണയം, ആർ ഹെച്ച് നിർണയം, രക്തസംക്രമിപ്പിക്കൽ വഴി പടരാവുന്ന രോഗ നിർണ്ണയം, പിന്നെ അപ്രതീക്ഷിത ആൻറിബോഡി നിർണ്ണയവും അത്യന്താപേക്ഷികമായ ഘടകമായ് പറയുന്നു.
ഗർഭാവസ്ഥ, രക്തസംക്രമിപ്പിക്കൽ, സൂചി പങ്കുവെയ്ക്കൽ, ചില രോഗങ്ങൾ, അജ്ഞാതമായ കാരണങ്ങൾ എന്നിവ മൂലം ശരീരത്തിൽ അല്ലോ ആന്റിബോഡികൾ ഉണ്ടാകുന്നു. ഇവയുടെ നിർണ്ണയം രക്തദാതാവിനു ഭാവി രക്തസംക്രമിപ്പിക്കൽ ഘട്ടങ്ങളിൽ ആന്റിജൻ ഇല്ലാത്ത സുരക്ഷിത രക്തം നേടിക്കൊടുക്കാനുള്ള അവസരം ഒരുക്കുന്നു .
ആട്ടോ ആന്റിബോഡികൾ ഉണ്ടാകുന്നത് ശരീരത്തിലെ തന്നെ ആൻറിജനുകളെ ശരീര പ്രതിരോധ ശക്തി ശത്രുവായ് ധരിക്കുന്നത് മൂലമാണ്. ഇത് ആട്ടോഇമ്മ്യൂൺ രോഗങ്ങളോ, പലവിധ മറ്റു രോഗാവസ്ഥകളിലോ കാണപ്പെടാം. ഇവയുടെ നിർണ്ണയം ആരോഗ്യമുള്ള ശരീരത്തിൽ കണ്ടുവരുന്നത് ഒരു അപായസൂചനയായേക്കാം. അതിനാൽ ആട്ടോ ആൻറിബോഡി പോസിറ്റീവായി കാണുന്ന ആരോഗ്യവാൻമാരായ രക്തദാതാവിനെ ഇത് അറിയിക്കുകയും അവർക്ക് നല്ല പ്രബോധനം നൽകി ഒരു ഫിസീഷ്യന്റെ അടുക്കലേക്ക് റഫർ ചെയ്യുകയും ചെയ്യും. ഈ പഠനം വഴി ചിലപ്പോൾ ഗുപ്തമായ രോഗങ്ങളെ വളരെ നേരത്തെ കണ്ടു പിടിക്കപ്പെടാനുള്ള ഒരു ഉപാധിയായിത്തീരാം. ചിലപ്പോൾ ഈ അപ്രതീക്ഷിത ആന്റിബോഡികൾ ഒരു തരത്തിലും ദോഷകരമല്ലാത്തതുമാകാം.

5. ഈ പഠനത്തിന്റെ വിധാനം എങ്ങനെ?
രക്തദാനത്തിനു ശേഷം പ്രാധമിക രക്ത ബാഗിലേക്കുള്ള ട്യൂബിൽ നിന്നുമുള്ള രക്തത്തിനെ ഗ്രൂപ്പിങ്ങ് , രോഗനിർണ്ണയം എന്നിവ ചെയ്യാനായി വിവിധ തരം ടെസ്റ്റ് ട്യൂബുകളിലേക്ക് മാറ്റുന്നു. ഇതിൽ നിന്നും ഒരു 2 മില്ലി രക്തസാമ്പിൾ ഇ. ടി .റ്റി. ഏ ടെസ്റ്റ് ട്യൂബിലേക്ക് പകരുന്നു. ഈ ടെസ്റ്റ് ട്യൂബിലെ രക്തമുപയോഗിച്ച് രണ്ടു തരം ടെസ്റ്റിലൂടെ ആട്ടോ ആന്റി ബോഡി / അല്ലോ ആൻറിബോഡി നിർണ്ണയം നടത്തുന്നു (ഡയറക്റ്റ് ആന്റിബോഡി ടെസ്റ്റ് / ഇൻ ഡയറക്റ് ആൻറിബോഡി ടെസ്റ്റ്).

6. ഈ പഠനം കൊണ്ട് നിങ്ങൾക്കുണ്ടാകുന്ന ഗുണം?
നേരത്തേ പറഞ്ഞത് പോലെ, ആട്ടോ ആന്റിബോഡി നിർണ്ണയം വഴി ഭാവി രക്തസംക്രമിപ്പിക്കലിനു സംപൂർണ്ണ സുരക്ഷിത രക്തം ലഭിക്കാൻ ഉപകരിക്കും. അതുപോലെ തന്നെ അല്ലോ ആൻറിബോഡി നിർണ്ണയം വഴി ഒളിഞ്ഞു കിടക്കുന്ന രോഗനിർണ്ണയത്തിനും ഉപകരിക്കാം. ഈ പഠനത്തിൽ പങ്കുകൊള്ളുന്നതു കൊണ്ട് നിങ്ങൾക്ക് ഒരു തരത്തിലും പണച്ചിലവ് വരുന്നില്ല. ആ ട്ടോ ആന്റിബോഡിയുള്ളവരെ നേരായ പ്രബോധനം നൽകി ഒരു ഫിസീഷ്യന്റെ അടുക്കലേക്ക് റഫർ ചെയ്യുന്നു.

7. ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നത് കൊണ്ട് നിങ്ങൾക്കുണ്ടായേക്കാവുന്ന അപായഹേതുക്കൾ എന്തൊക്കെ?
ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നത് മൂലം നിങ്ങൾക്ക് ഒരു തരത്തിലുള്ള അപായസാദ്ധ്യതകളുമില്ല. എന്നാൽ രക്തദാനം ചെയ്യുന്ന വേളയിൽ വിളർച്ച തളർച്ച മുതലായ അസ്വസ്ഥതകൾ അനുഭവപ്പെട്ടെന്നിരിക്കാം. അവയെല്ലാം രക്ത ബാങ്കിലെ വിദഗ്ദരായ ഡോക്ടർമാരും പരിശീലനം നേടിയ മറ്റു പ്രഗത്ഭരായ സ്റ്റാഫുകളും നല്ല പരിചരണയും ശുശ്രൂഷയും നൽകി തത്സമയം ഭേദപ്പെടുത്തുന്നതാണ്.

8. ഈ പഠനം വഴി സമൂഹത്തിനുള്ള ഗുണങ്ങൾ?
ഈ പഠനം വഴി പ്രസ്തുത ജനസമൂഹത്തിലെ അപ്രതീക്ഷിത ആൻറിബോഡികളുടെ പ്രാവീണ്യത്തെക്കുറിച്ചും അറിയാൻ കഴിയും. ആരോഗ്യവാൻമാരായ രക്ത ദാതാക്കളിലെ ഗുപ്തമായ രോഗ നിർണ്ണയത്തിനും വഴിതെളിക്കുന്നു. കൂടാതെ ഭാവി രക്തസംക്രമിപ്പിക്കൽ ആവശ്യകതകൾക്ക് സുരക്ഷിത രക്തം കൈവരിക്കുവാൻ പ്രയോജനപ്പെടുന്നു . സമൂഹത്തിലെ അപ്രതീക്ഷിത ആന്റിബോഡി ആധിക്യത്തെ മനസ്സിലാക്കുന്നത് വഴി ഈഇൻസ്റ്റിട്ട്യൂട്ടിലെ എല്ലാ രോഗികൾക്കും ഉത്തമ സുരക്ഷിത രക്തസംക്രമിപ്പിക്കൽ പ്രക്രീയകളെ ഉയർത്തിക്കൊണ്ടു വരാനും സഹായിക്കുന്നു. നമ്മുടെ രാജ്യത്തിൽ ഇത്തരമൊരു പഠനത്തിന്റെ വിരളത കണക്കിലെടുത്ത് ഇത് സമൂഹനന്മയ്ക്ക് ഉതകുമെന്നും കരുതുന്നു.

9. ഈ പഠനത്തിൽ നിന്നും എനിക്ക് എപ്പോളെക്കെ പിൻമാറാനുള്ള അവസരമുണ്ട്?
നിങ്ങളുടെ പങ്കാളിത്തം തികച്ചും സ്വേച്ഛാനുസാരമാണ്. ആയതിനാൽ പഠനകാലയളവിൽ ഡാറ്റ ശേഖരണവേളയിൽ എപ്പോൾ വേണമെങ്കിലും കാര്യകാരണ വിശദീകരണം നൽകാതെ തന്നെ ഈ പഠനത്തിൽ നിന്നും പിൻമാറാനുള്ള പൂർണ്ണ അവകാശം നിങ്ങൾക്കുണ്ട്. പിൻവാങ്ങൽ കാരണം നിങ്ങൾക്കു തരുന്ന വൈദ്യ സഹായത്തിനോ നിയമാനുസൃതമായ അവകാശങ്ങളേയോ യാതൊരു തരത്തിലും പ്രതികൂലിക്കുന്നില്ല.

10. നിങ്ങളുടെ വ്യക്തിപരമായ വിശദാംശങ്ങളുടെ സ്വകാര്യത എങ്ങനെ കൈകാര്യം ചെയ്യുന്നു?
ഈ ഗവേഷണം അംഗീകൃതമായി കഴിഞ്ഞാൽ ഇത് ഒരു പ്രബന്ധമായോ അല്ലെങ്കിൽ ഒരു മെഡിക്കൽ പ്രസിദ്ധീകരണത്തിലൊ പ്രകാശിപ്പിച്ചേക്കാം. എന്നാൽ നിങ്ങളുടെ വ്യക്തിപരമായ യാതൊരു വിശദാംശവും എവിടെയും പരാമർശിക്കുകയില്ല. പക്ഷെ വ്യക്തിപരമായ വിശദാംശങ്ങളും മറ്റു ഡാറ്റകളും പിൻവാങ്ങിയാൽ ക്കൂടിയും ഇൻസ്റ്റിട്ട്യൂട്ട് നീതിശാസ്ത്ര കമ്മിറ്റി അംഗങ്ങൾക്കും പഠനവുമായ ബന്ധമുള്ള കാര്യനിർവ്വഹണ സംഘാംഗങ്ങളൾക്കും നിങ്ങളുടെ അനുവാദം കൂടാതെ പരിശോദിക്കാനുള്ള അവകാശമുണ്ട്.

ഇതു സംബന്ധിച്ച് ഏത് സംശയ നിവാരണത്തിനും എന്നെയോ എന്റെ കോ ഗൈഡിനെയോ ബന്ധപ്പെടാവുന്നതാണ്.
ഞാൻ ഡോ.ഗായത്രി എ.എം.
ഫോൺ: 04712524636/9446208632
ഇ-മെയിൽ: gayathri@sctimst.ac.in
അല്ലെങ്കിൽ
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പ്രധാന നിരീക്ഷകന്റെ പേര്:
ഡോ.ഗായത്രി എ.എം
വിലാസം: ജൂനിയർ റസിഡന്റ്
ട്രാൻസ്ഫ്യൂഷൻ മെഡിസിൻ ഡിപ്പാർട്ട്മെന്റ്
ശ്രീ ചിത്ര തിരുന്നാൾ ഇൻസ്റ്റിട്ട്യൂട്ട് ഫൊർ മെഡിക്കൽ സയനൻസസ് ആൻറ് ടെക്നോളജി, തിരുവനന്തപുരം
ഫോൺ: 04712524636/9446208632

പ്രധാന നിരീക്ഷകന്റെ ഒപ്പ്:
ഡേറ്റ്:

നീതിശാസ്ത്രപരമായ സംശയങ്ങർക്ക് വകുപ്പ് മെമ്പർ സെക്രട്ടറി ഡോ.മാലാ രാമനാഥനെ ബന്ധപ്പെടാവുന്നതാണ്.
ഫോൺ: 04712524234
ഇ-മെയിൽ: iec.mem.sec@sctimst.ac.in

വിവരങ്ങൾ വ്യക്തമാക്കിയുള്ള സമ്മതപത്രം

പഠനതലക്കെട്ട്: ദക്ഷിണ കേരളത്തിലെ ഒരു മൂന്നാം വിഭാഗത്തിൽ പെട്ട ആശുപത്രിയിലെത്തുന്ന രക്തദാതാക്കളുടെ ചുമന്ന രക്താണുക്കളിലുള്ള അപ്രതീക്ഷിത ആൻറിബോഡികളുടെ ആധികൃത്തെക്കുറിച്ചുള്ള പഠനം

പഠന നമ്പർ:_____

പങ്കെടുക്കുന്നയാളുടെ ക്രമ നമ്പർ: _____

ജനന തിയതി: _____

ഇന്നത്തെ തിയതി : _____

_____ തിയതിയിലുള്ള ഈ പഠന വിവരണ പത്രിക ഞാൻ പൂർണ്ണമായും വായിച്ചു മനസ്സിലാക്കുകയും ഇത് സംബന്ധിച്ചുള്ള ചോദ്യങ്ങൾ ചോദിക്കാനുള്ള അവസരവും ലഭിച്ചു. ഈ പഠനത്തിൽ എന്റെ പങ്കാളിത്തം തികച്ചും സ്വേച്ഛാവഹം ആണെന്നും എപ്പോൾ വേണമെങ്കിലും സ്വയേഷ്ടം കാര്യകാരണ വിശദീകരണങ്ങൾ നൽകാതെ തന്നെ പഠനത്തിൽ നിന്നും പിൻമാറാനുള്ള സർവ്വ സ്വാതന്ത്ര്യം എനിക്കുണ്ടെന്നും അത് മൂലം എനിക്കു തരുന്ന വൈദ്യ സഹായത്തിനോ നിയമാനുസൃതമായ അവകാശങ്ങളേയോ യാതൊരു തരത്തിലും പ്രതികൂലിക്കുന്നില്ലയെന്നും മനസ്സിലാക്കുന്നു. കൂടാതെ എന്റെ വ്യക്തിപരമായ വിശദാംശങ്ങളും മറ്റു ഡാറ്റകളും ഞാൻ പിൻവാങ്ങിയാൽ കൂടിയും ഇൻസ്റ്റിട്ട്യൂട്ട് നീതിശാസ്ത്ര കമ്മിറ്റി അംഗങ്ങൾക്കും പഠനവുമായ ബന്ധമുള്ള കാര്യനിർവ്വഹണ സംഘാംഗങ്ങളൾക്കും എന്റെ അനുവാദം കൂടാതെ പരിശോദിക്കാനുള്ള അവകാശമുണ്ടെന്ന് മനസ്സിലാക്കുന്നു. അതിനു എന്റെ പൂർണ്ണ സമ്മതം അനയിച്ചുകൊള്ളുന്നു. എന്നാലും എന്റെ വ്യക്തിപരമായ വിശദാംശങ്ങൾ ഒരു തരത്തിലും പ്രസിദ്ധീകരിക്കില്ലെന്നും മൂന്നാമതൊരാൾക്ക് കൈമാറില്ലെന്നും ഞാൻ മനസ്സിലാക്കുന്നു. ഈ പഠനത്തിൽ നിന്നും ഉളവെടുക്കുന്ന ഏതൊരു വിവരമോ / ഫലത്തേയോ അതിന്റെ ശാസ്ത്രീയമായ രീതിയിലുള്ള ഉപയോഗത്തിനു ഒരിക്കലും തടസ്സം നിൽക്കുകയില്ല. ഈ പഠനത്തിൽ പങ്കെടുക്കാൻ എനിക്ക് പരിപൂർണ്ണ സമ്മതമാണ്.

പങ്കെടുക്കുന്ന ആളിന്റെയോ / നിയമപരമായ് സ്വീകാര്യമായ പ്രധിനിതിയുടെ ഒപ്പ് / വിരളടയാളം

നിരീക്ഷകന്റെ ഒപ്പ്:

തിയതി :

PROFORMA

Donor unit no: Sex: M / F

Date: Age:

DOB: Address:

Marital Status:

Contact no: Blood Group:

Occupation:

E-mail:

Participant No:

- First time/ Repeat: _____ Mention if you are a **G-DONOR**: Y / N
- If repeat, how many times: _____
- Date & place of last donation: _____
- Any history of previous blood or blood component transfusion:
- If yes, how many units transfused:
- How long back last transfusion occurred: _____
- History of pregnancy/ abortion (if female donor):
- History of any hospitalisation in past (if yes, mention):

- History of needle sharing:
- Are you on any medication currently?
- Are you in any sort of treatment for any medical condition? (if yes, mention):

- Any significant past medical history (if yes, mention):

- Any history of medical conditions/malignancy in family:

Laboratory Reports

- Blood group:
- DAT results: Positive / Negative
- Antibody detection (Donor Serum + pooled O cell 3-5% suspension):
  - At 4^0C:
  - 22^0C:
  - 37^0C:
  - IAT:
- Auto-control (Donor Serum + Donor cell 3-5%suspension):
  - At At 4^0C:
  - 22^0C:
  - 37^0C:
  - IAT:
  - Enzymes:
- Antibody Identification:
- Titre of antibody: IgM _____ IgG _____

MASTER SHEET

Sl no.	Date	Donor ID	Age	Sex	Blood Group	Type of Donor	DAT	Auto-control			O Cells			Pap O cells	Antibody Identified	Type of Antibody	Titre	
								4^0	22^0	37^0 (AHG)	4^0	22^0	37^0 (IAT)				IgM	IgG
1	12/02/2018	170024	19	M	A neg	First Time	0	+	0	0	+	+	+	+	Anti-IH	IgM+IgG	64	2
2	02/03/2018	170183	34	M	A neg	First time	0	+	0	0	+	+	+	+	Anti-Le^b	IgM+IgG	32	8
3	24/05/2018	170272	34	M	B +	Repeat	0	0	0	0	+	+	+	0	Anti-M	IgM+IgG	64	16
4	25/07/2018	172226	35	F	AB neg	First Time	0	0	0	0	0	0	+	+	Anti-D	IgG	-	32
5	17/09/2018	173235	26	M	B +	First Time	0	+	0	0	+	+	+	+	Anti-Le^{ab}	IgM+IgG	64	8
6	28/10/2018	173878	32	M	0 +	Repeat	0	0	0	0	0	+	+	0	Anti-M	IgM+IgG	32	16
7	31/01/2018	174954	29	F	B neg	Repeat	0	0	0	0	0	0	+	+	Anti-C	IgG	-	8