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RBC GENOTYPING FOR IMPROVED OUTCOMES IN CHRONIC TRANSFUSION PATIENTS

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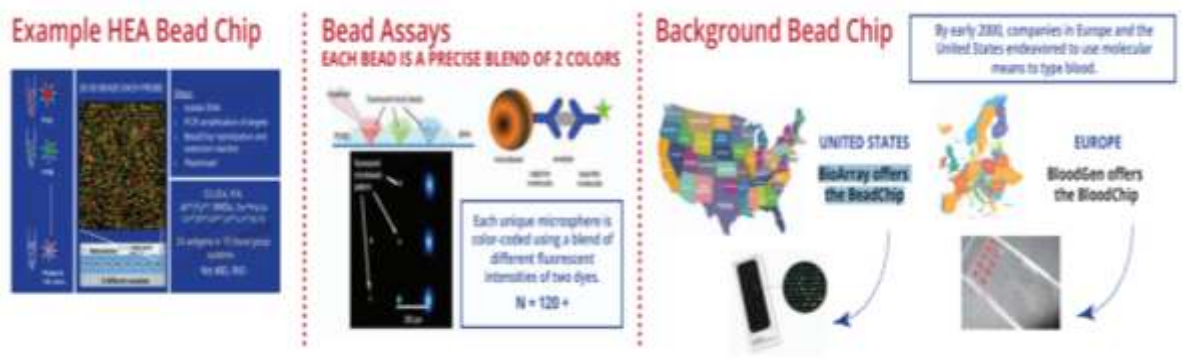
ABSTRACT

Blood transfusions are often an important component of medical care for many patients. We studied the differences between classical phenotyping and compared the results with the RBC genotyping using the Beadchip technology. There were 40 patient specimens involved in this study. It was co ncluded that the RBC genotyping is the far superior and accurate method to provide the best transfusion care to patients.

INTRODUCTION

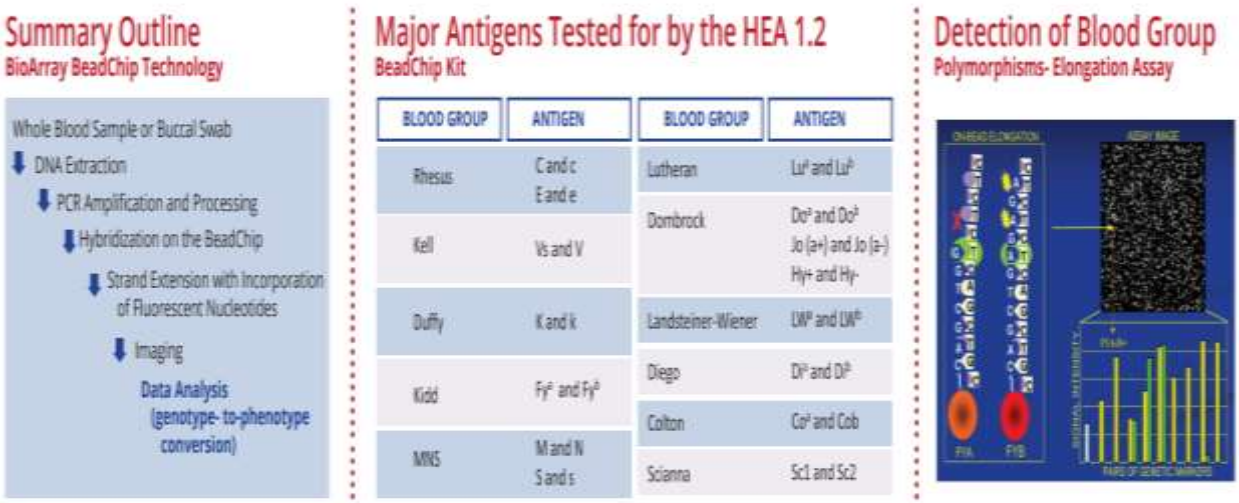
Blood transfusions are an essential medical procedure for many patients. A blood transfusion occurs in the U.S. every two seconds. (Jefferson M. Jones, 2022) Blood carries oxygen and nutrients to living cells and takes away their waste products. It also delivers immune cells to fight infections and contains platelets that can form a plug in a damaged blood vessel to prevent blood loss. (Dean L., 2005) In patients over the age of 64, transfusion of blood and blood products ranks as the second most common procedure performed in U.S. hospitals. For patients between the ages of 45-64, it is the fifth most common procedure. Overall, transfusion of blood and blood products occurs in 3.5-5.1 percent of hospital stays, depending on patient age group. (Kamille A. West, 2016) Fresh whole blood has always been thought of as the standard for transfusion. Modern medical advancements have allowed the efficient use of the different components of whole blood, such as red blood cells (RBCs), individual factor concentrates, fresh frozen plasma (FFP), platelet concentrates, and cryoprecipitate. (Lotterman S, 2022) For patients receiving blood transfusions the collaboration between laboratory and clinical medicine professionals, and physicians from multiple specialties, such as pathology and hematology is vital for the safety of the patient. There can be adverse reactions to blood transfusions. The most common signs and symptoms include fever, chills, hives, and itching. Other symptoms may indicate a more serious reaction, such as: respiratory distress, high fever, hypotension, and hemoglobinuria. (Suddock JT, 2023) Alloimmunization is defined as an immune response to foreign antigens after exposure to genetically different cells or tissues. It is one of the major complications of regular blood transfusions, particularly in patients who are chronically transfused. (Mota MA, 2013)



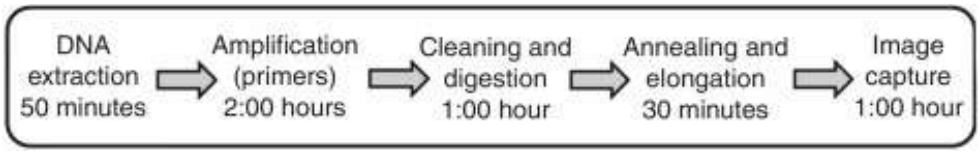
The detection of blood group antigens is essential in transfusion practice to prevent alloimmunization. Blood group systems are characterized by the presence or absence of antigens on the surface of erythrocytes and are genetically distinct. Currently, there are 43 blood group systems containing 345 antigens for human RBCs recognized by the International Society of Blood Transfusion. (Gassner C, 2022) The probability of an

individual producing one or more anti-erythrocyte antibodies is approximately 1% per unit of blood transfused, and in chronically transfused patients the alloimmunization rate may reach 50%. Blood typing by antibody-based methods has been the standard for determining ABO, Rh, and "extended" blood group antigens present on red blood cells. The antigens expressed on the red blood cell determine an individual's blood group. The main two blood groups are called ABO and classifies blood types as A, B, AB, and O in conjunction with the Rh classification including Rh D-positive or Rh D-negative blood types. (Dean L., 2005) The Rh blood group includes the RHD and RHCE genes, that are closely linked. The genes encode for 49 known Rh antigens. (Dean L., 2005) Other blood group systems tested in this study include the Kidd and Kell blood groups. The Kell blood group system contains the third most influential antigens following the ABO and Rh groups respectively. (Dean L., 2005) The antibodies that target these blood groups antigens can cause transfusion reactions and hemolytic disease of the newborn. (Dean L., 2005) The identification of these blood groups is vital for treatment. The primary method of blood phenotyping is blood agglutination or hemagglutination. Agglutination occurs when antibodies bind to specific binding sites on antigens expressed on red blood cells (RBCs). (Samae M, 2021) Hemagglutination has certain limitations including: the patient's red blood cell antigens cannot be reliably phenotyped due to recent history of receiving blood transfusion; there are prior or significantly interfering drugs (such as anti-CD47); it cannot evaluate genetic changes such as partial antigens that may explain alloantibodies but positive phenotyping; and in rare cases when uncommon antibodies form and antigen profiling beyond the common antigens mentioned is required. (Reid ME, 2009) This work explores the role of molecular blood typing techniques, via the Beadchip technology, in reducing the risk of alloimmunization, the potential for identifying uncommon antigens in transplant cases, and decreasing unnecessary usage of Rh prophylaxis in prenatal medicine.

Procedure



DNA samples from 40 multi-transfused patients including thalassemia and sickle cell disease were used for red blood cell genotyping. The genotyping will be performed using the human erythrocyte antigen (HEA) BeadChip™ system manufactured by BioArray Solutions (Immucor, Warren, NJ). The (HEA) BeadChip™ system requires whole blood specimens collected into EDTA anticoagulant tubes. The DNA from these samples is extracted via the QlAamp DSP DNA Blood Mini Kit (QIAGEN cat# 61104) following manufacturer's instructions for use. The extracted DNA is then amplified via polymerase chain reaction (PCR). Following multiplex PCR amplification, the DNA requires two post-PCR processing measures: amplicon clean-up and single-stranded target generation. The post-PCR measures use Clean-up Reagent and Lambda Exonuclease to produce a single-stranded DNA that are incubated on the BeadChip array. The incubation on the BeadChip array allows elongation of the DNA strand to combine fluorescently-labeled dNTP molecules only on those probes where the 3' end exactly matches the annealed DNA. The BeadChip array holds a library of individual bead types that contain all of the probes of interest. Each probe is covalently bonded to a spectrally distinguishable bead type thus allowing for the capturing of fluorescent signals from individual beads simultaneously. The BioArray Array Imaging System determines the identity of the bead by the color of the bead, its position in the array, and report the average signal intensity, coefficient of variance standard deviation of the intensities, and number of beads measured for each type of probe. This information is further analyzed by the HEA Analysis software in BioArray Solutions Information System to generate assay results. The results from the BeadChip genotyping will be compared with previously determined phenotyping results for RhD, RhCcEe, Kell, and Kidd by hemagglutination method. (Paccapelo C, 2015).



RESULTS

The result of the genotyping showed some discrepancies between the results of the hemagglutination methods among the patients. Out of the forty patients tested, fifteen patients had discrepancies between genotyping and phenotyping results in a total of 25 alleles. The discrepancies occurred in all three phenotyping systems. The charts below shows the Rh, Kell, and Kidd alleles associated with the hemagglutination method for phenotyping red blood cells. The highlighted numbers show the alleles where discrepancies have occurred. In 12 patients, the discrepancies had the potential of alloimmunization.

Rh System	RhD+	RhD-	Rh C+c−	Rh C+c+	Rh C−c+	Rh E+e−	Rh E+e+	Rh E−e+
RHD +*	31							
Partial *RHD**	2							
RHD −*		5						
*RHCE*CC*			3	2				
*RHCE*Cc*				18				
*RHCE*cc*					15			
*RHCE*EE*						2	1	
*RHCE*Ee*							10	1
*RHCE*ee*							2	22

Kell system	K+k−	K+k+	K−k+
*KEL*01/KEL*01*			
*KEL*01/KEL*02*		3	1
*KEL*02/KEL*02*			34

Kidd system	Jk(a+b−)	Jk(a+b+)	Jk(a−b+)
*JK*01/JK*01*	9		
*JK*01/JK*02*	1	21	
*JK*02/JK*02*		2	5

CONCLUSION

Blood transfusions are often an important component of medical care for many patients. Currently, the gold-standard for typing RBCs is hemagglutination in which blood group systems are characterized by the presence or absence of antigens on the surface of erythrocytes. The use of PCR based assays in blood group genotyping has vital importance in transfusion management of chronically transfused patients. This method is especially useful if the patients were not phenotyped prior to starting the initial transfusions. The results showed fifteen out of the 40 or 38% of patients had discrepancies between genotyping and phenotyping results. From the results of our study the RBC genotyping leads to more specific results for the patients reducing potential for alloimmunization. The use of RBC genotyping can have provide future benefits in: Fetal DNA typing; Extensive blood group typing of donors for alloimmunized patients; determining the blood group of a recently transfused patient; screening blood donors to find rare blood group phenotypes; determining the frequency of blood group polymorphisms in a population; determining RHD zygosity for fathers of fetuses at risk for HDFN; and blood group typing of patients with autoimmune hemolytic anemia. Within our study of these forty patients RBC genotyping is the far superior and accurate method to provide the best transfusion care to patients. (Westhoff, 2019)

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