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Production of ABO Anti-Sera and A Slide and Filter Paper-Base Technique for Abo Blood Grouping Using Rejected Blood

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ABSTRACT

ABO is the most important blood group system in transfusion medicine and transplantation immunology. The ABO blood groups differ by the presence or absence of antigens on red blood cells surfaces and antibodies in plasma. Accurate determination of ABO blood group system is critical. The determination of this ABO status is based on some traditional serological techniques. Food and oxygen are supplied to the cells and metabolic by-products exported from the same cells by blood. Anemic patients require blood transfusion to be able to meet up with their normal cellular function. Blood is yet to be produced artificially and some of its components can only be stored for a short period of time. To meet patients need for blood, a wide and healthy base of blood donors, willing to donate blood when required, are needed. The donated blood sometimes could be unsafe for transfusion and in such cases, should be rejected and discarded. This study therefore was aimed at recycling this rejected blood in the healthcare system to make it useful again by developing a slide and filter paper-based test for ABO blood group system. Red blood cell membrane containing antigens A and B from blood group A and B respectively were isolated and these antigens used to immunize animals. Few months later the animals were bled and anti-sera against these antigens collected. The anti-sera were used to test for ABO compatibility on the slide and filter paper. When the anti-sera was tested for antibody production the results was negative with non-immunized animals and positive for animals that were immunized. When the filter paper strip was used for blood grouping the blood stayed almost at the same spot for strip immobilized with non-compatible anti-sera

Keywords; Antigen, anti-sera, Rejected-Blood, production, ABO Blood grouping system

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INTRODUCTION

Food and oxygen are supplied to the cells and metabolic by-products exported from the same cells by blood (Nwabueze *et al*., 2014, Mulcahy *et al.,* 2016, Nureye *et al*., 2019). Anemic patients require blood transfusion to be able to meet up with their normal cellular function. Transfusion medicine is therefore an important component of the healthcare system of all countries (WHO, 2017). The hemoglobin concentration, the amount of blood loss and the clinical condition of patients are the basic determinant factors for red blood cell transfusion. The main therapeutic strategy in the treatment of acute hemorrhage is to prevent or correct hypovolemic shock in other to ensure tissue oxygenation. It is also important to restore circulatory volume by infusing crystalloid sufficient amount to maintain a satisfactory blood flow and blood pressure (Giancarlo *et al*., 2009). One of the events that brought transfusion medicine forward was the discovery of the ABO system by Karl Landsteiner. This opened the door for performing safe blood transfusion. Before the discovery of this system, many transfusions often lead to death due to incompatibility (Emili *et al*., 2012). In other to avoid this transfusion complications the correct grouping of human blood into the ABO blood system is imperative for numerous reasons among them transfusion compatibility. The ABO blood systems are therefore of primary importance when transfusing blood, as incompatibility may lead to an acute hemolytic reaction with catastrophic results such as shock and renal failure leading to death. Without ABO compatibility testing, around one third of the unscreened blood transfusions would be expected to cause a hemolytic reaction. For this reason blood should be type for ABO identification and cross match for compatibility before any transfusion (Mayank et *al.,* 2012).To meet patients need for blood, a wide and healthy base of blood donors, willing to donate blood when required, are needed. Blood is yet to be produced artificially and some of it components can only be stored for a short period of time (Lorenz and Alois, 2019).Voluntary, unpaid and safe blood donation is an important aim for the prevention of blood scarcity. However this is not often the case especially in low and middle-income countries where most of the donation is done by relatives or friends of the recipient and paid donors (Dora *et al*., 2001, Nureye et al., 2019). The donated blood, sometimes maybe unsafe for transfusion. In 2001 Doral et al demonstrated that an average of about 20% of donated blood was rejected each year in Yaoundé-Cameroon due to positive immunodeficiency virus (HIV) and or hepatitis B antigen. This study therefore is aimed at recycling the rejected blood into the health care system by using it to develop antisera and a filter paper based prototype which can be used for ABO compatibility testing. Also to ensure that the effort of the donors to safe life's don't go in vain.

MATERIALS AND METHOD

Choice of animals

The selection of the animal species for the production of antibody depended on, the amount of antiserum needed, the ease of obtaining blood samples or bleeding the animals as well as the cost of the specie (Leenaars *et al*., 1999). From this rabbits were chosen though more expensive as compared to mice, rats, and guinea pigs, they were found to have some advantages over the other species. For instance there are easy to bleed, easy to immunize and boost a higher immune response as compared to mice, rat, and guinea pigs. Mice and rat take a longer time to response to immunization. Other species like goats and horses could not be used as they are far more expensive.

Sampling

Whole rejected blood samples were gotten from the Yaoundé[1] university teaching hospital (CHU) blood bank. The blood was transported on ice blocks in a cooler to the laboratory and stored at +4 ^{o}c.

Preparation of immunogen

10mL of Blood group A and B were respectively introduced into 50mL tube and the volume completed to 40mL with an isotonic buffer (0.9% NaCl; 5Mm Na2PO4, PH8) to isolate antigen A and B respectively. The buffer prevented the red blood cells from swelling, shrinking and or crenating. This mixture was thoroughly mixed to begin the process of washing the red blood cells from plasma proteins. The sample mixtures were then centrifuged at 4000 rotations per minute (rpm) for 30 minutes at 4°c. The mixtures then separated into a red blood cell pellet at the bottom and a plasma supernatant at the top. The red blood cells spin down owing to their high hemoglobin concentration. The supernatants were then separated from the blood pellet with the help of a micropipette. The blood pellets were re-suspended in the isotonic buffer for the second and third time and centrifuge at 4000 rotations per minutes (rpm) for 30 minutes at 4°c. This second and third wash was to ensure that almost all of the plasma proteins were completely eliminated from the pellets. We therefore referred to this step as the wash step. At each wash step the supernatants were separated from the red blood pellet.

After two to three centrifugation or "spins," the buffy layer containing white blood cells were lost, and platelets will not have spun down as quickly as red cells, so the pellet consisted almost exclusively of red blood cells. The red blood cells pellets were then suspended in a hypotonic buffer (5mM NaPO2, PH8, without NaCl) to lyse most of the red blood cells. The whole red cells produced a cloudy, opaque suspension. The lysed mixtures were then centrifuged at 4000 rpm for 30minutes at 4 °c. The supernatants were separated from the pellets using a micropipette. The blood pellets were re-suspended in the hypotonic buffer for the second and

third time and centrifuged at 4000 rpm for 30 minutes at 4 °c. The supernatants were again separated from the pellet using a micropipette. The pellets were then suspended one last time in an isotonic buffer and then physiological buffer. At each suspension, the mixtures were centrifuge and supernatant separated as stated above. The pellets were then gently agitated to liquefy and conserved at +4 °c for subsequent immunization.

Animal preparation and immunization

The hind limb and the left ear of the animal where shaved using a scissors. This allowed a clear visualization of the site of injection and clear work place. To avoid invasions by microorganisms the limb and the ear were aseptically scrubbed and rinsed with 70 % alcohol. The marginal ear was flicked several times to allow the vein to dilate. The alcohol was then allowed to evaporate. The pellets were removed from +4^{o}c and the temperature allowed to change to room temperature before immunization. With the help of 2ml needle 0.5ml of the isolate were injected intravenously and intramuscularly respectively. The injected site of the animals were assess for tissue necrosis and abscess formation. The activities of the animals were also evaluated through the amount of water consumed, and their weights. Another group of animals were used as control and were never immunized. The animals were re-immunized in a two-week interval for a period of three months to boost the immune system.

The animals were bled following a protocol adapted in accordance to the healing center for wild life and blood sampling (Medaille ***et al.,*** 2005). The animals were bled at the end of each month for a period of 3 months to test for antibody production. A final bleed was done during the last month. After each bleeding the blood was centrifuges at 2000 rpm for 15 minutes.

The supernatant was separated from the pellets. Taking care not to disturb the cell layers. The serum was inspected for turbidity. In case of turbidity the serum was again centrifuged.

Testing serum for antibodies

At each bled the serum was tested for antibody production as follows; 10µl of serum from none immunized animal was introduced on to two points on a glass slid. Blood group A was then mixed with one of the serum and Blood group B with the other. They were both observed for agglutination. The same was done with serum from non-immunized animals.

Production of prototype

We cut filter papers (3cmx5.5cm). 10mlx10ml fingers (strips) like projections were made on the filter paper. Antibodies were immobilized on the filter paper strips and inoculated at different time. The filter papers were allowed to dry for, 10minutes, 20minutes, 30minutes, one hour and two hours in a hood. Others were kept at +4°c for one day, two days three days, one week and two weeks, after each incubation time 2µl of blood was introduced to the strips and observed. In case of poor observational difference the blood samples were further washed with 15µl of physiological buffer.

RESULTS AND DISCUSSION

Assessing serum for antibody production

No agglutination was observed for serum obtained from control animals tested with blood group A and B. The serum from animals immunized with antigen A revealed an immediate agglutination with blood group B after testing for antibody production with blood group A and B. However, a light agglutination was observed with blood group A after a few minutes The serum from animals immunized with antigen B revealed an immediate agglutination with blood group A after testing for antibody production with blood group B and A. However, a light agglutination was observed with blood group B after a few minutes.

Evaluation of prototype with produced antibodies

When the strips were tested with blood group A, at the strip immobilized with antibody A, the blood was found to migrate slowly and then stayed on the same point of deposition. On the strip immobilized with B the blood immediately spread out. When the strips were tested with blood group B, at the strip immobilized with antibody B the blood was found to migrate slowly and then stayed on the same point of deposition. On the strip immobilized with A the blood immediately spread. When the strips were tested with blood group O, the strip immobilized with A and B the blood immediately spread out even before it was wash with saline.

DISCUSSION

We aimed at isolating red blood cells membrane containing antigens from blood groups A and B, using this isolated antigens for the production of antibodies (anti sera) and then immobilizing the antibodies on strips of filter papers to produce a rapid diagnostic test for ABO blood group system or to produce a prototype to be used as a rapid test for the ABO blood group system. The productions of the antibodies were confirmed by testing serum isolated from control animals and immunized animals. Whenever the serum isolated from the control animals was tested for antibodies production, the test was always negative. That is no agglutination was observed for blood groups A and B. when the serum from immunized animals were tested the test was always positive. That is When serum from the animals that were immunized with antigen A, was tested for antibody production with blood group A and B, agglutination was immediately observed with blood group B. with a light agglutination observed with blood group A some minutes later When serum from the animal that was immunized with antigen B, was tested for antibody production with blood group B and A, agglutination was immediately observed with blood group A. with a light agglutination observed with blood group B some minutes later. This was similar to what was obtained by Bamoleke ***et al., 2012***) though they did not make use of isolated antigens for the production of anti-sera. They had a better length of agglutination than ours owing to their longer immunization period and the adjuvant they

used during immunization. However we later on noticed that most of the lysed cells were aspirated alongside the supernatant since membrane of red blood cells do not pack closely. This also could have possibly accounted for the difference in our results. The results obtained with the strips were similar to those obtained by Mohammad et al in 2011 and Temsiri et al in 2018 where after immobilizing antibodies on filter and inoculating them with erythrocytes. They watch with 100ul PBS. Where they was no agglutination the erythrocytes where completely wash away.

CONCLUSION

Antigens A and B on the membrane of red blood cells were used to produce anti-sera in rabbits. These anti-sera were used for ABO compatibility testing using the slide and filter paper (prototype) based test.

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