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THE APPLICATION OF ANTIBODY COATED PAPER STRIPS IN BLOOD TYPING

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Abstract

This project was designed to develop a paper based rapid ABO blood typing method. The paper was soaked in antisera A and antisera B to trigger agglutination when blood droplets were placed on the treated paper. Hence, three objectives were analysed in this experiment. The first was about coating the antibodies onto the paper strips. All the paper strips were soaked into antibody solutions of different concentrations. Secondly, the demonstration of typing ABO blood groups by using the antibody coated paper strips. Thirdly, the determination of the strength of blood agglutination with the antibody coated paper strips. The data was analysed as descriptive data. A wicking distance determines the strength of agglutination. Each box in the strip represented four (+), three (+), two (+) and one (+) from the centre to either direction. A positive result demonstrated the strongest agglutination that graded four (+) while a negative result demonstrated to be the weakest agglutination that graded one (+) for all four types of blood groups. The occurrence of blood chromatographic separation is based on the specific antibody- antigen interactions. Blood agglutination and wicking action on the paper strips followed the concepts of colloids chemistry. The immunoglobulin M antibodies agglutinated the red blood cells by polymer bridging, upon selective adsorption on the specific antigen at their surface.

Keywords: ABO blood typing, antibody, antigen

1 INTRODUCTION

ABO blood group system is vital in determining type of blood groups. Blood group typing is a technique that is used to determine specific type of blood group a person belongs. Human blood is varies due to the present and absence of antigens and antibodies. Antigens are on the surface membrane of the red blood cells whereas antibodies derived from plasma. Each person varies in types and combination of these certain protein molecules. It is a standard practice to test for A, B, and D (Rh) antigens. ABO blood group typing is confirmed by reverse grouping that detects expected isoagglutinins (Behra, D. R., & Joshi, D. Y. R., 2013). The test for blood grouping is known as the slide test and it is done in laboratories manually. Natively, most of the methods applied are yet based on the principle of interaction between antigen and antibody following agglutination of the red blood cells which indicate a positive result. Negative result shows when there is no agglutination takes place due to poor interaction. This manual blood grouping test leads to major mistakes such as wrong blood group classifications and may cause typing error in the report (Behra, D. R., & Joshi, D. Y. R., 2013). There are various blood typing methods which are also applicable by advanced equipment such as, gel column, thin-layer chromatography (TLC), immunostaining, fiber optic-microfluidic device, spin tube method and many more. Among those, the recognition and automation of red blood cell (RBC) agglutination by antigen-antibody interaction often needs optical or microfluidic analytical equipment which are not affordable in most third world countries. Thus, a cheaper yet effective paper based instantaneous blood typing technique can be a substitute. The ability of materials to absorb fluids like red cells using paper that consist of porous media can be used to view the agglutination of red cells. Development of economical paper based diagnosis for blood typing can be demonstrated as it enables the visibility of agglutinations. Currently, no handy budget disposable tests accessible for 'on the spot' study of blood type (Sood, 1999). Furthermore, this method would be more beneficial and efficient especially for a mobile blood donation campaign.

2 EXPERIMENTAL SECTION

Antibody solutions of red cell antigens A, B, and D (Epiclone Anti - A, Anti-B, and Anti-D) were used. Anti- A was in blue color reagent, Anti-B was in yellow color reagent and Anti-D was a clear solution. Anti-D was agglutinated with any blood (A, B, O) with the Rhesus factor (D). Phosphate buffer saline (PBS) (Invitrogen) and it was used as diluent for all antibody solutions. Two centimeters wide paper strips known as Whatman filter paper grade four were purchased and was used as porous substrate for antibody. A HP Deskjet 4515 was used to print two centimeter units on each paper strip and this was used to measure the wicking distance. Standard blotting papers were purchased and were used to remove the excess of antibody solution from the treated paper strips. Reflex paper was purchased and was used as a semi hydrophobic surface. A calibrated micropipette was used to dispense standard (20μL) droplets on the paper strips. Expired blood samples obtained (A, B, AB and O) from iHeal Medical Centre and

KLCC Medical Centre. All the antibody and blood samples were stored below 4°C.

2.1 Slide ABO Test.

Each blood group sample was tested with Slide ABO test to determine the type of blood group of each EDTA tube and each EDTA tube was labeled with respective blood groups. A 10% of suspension in saline was prepared (Khan *et al*, 2010).

2.2 Antibody coating

Paper strips were soaked into antibody solutions of different concentrations (Anti-A at 1.0x, 0.8x, 0.6x, 0.4x, 0.2x and 0.0x) and each concentration was replicated six times. Phosphate buffer saline was used as diluent. Excess antibody was removed from the paper strips with blotting paper. The antibody (Anti-A) active paper strips was placed on Reflex Paper. Blood droplets of 20µL were dispensed at the centre of the paper using a calibrated micropipette. The wicking distance was observed from the centre to either direction and graded accordingly (Czajkowsky, Daniel M., and Zhifeng, S., 2009).

2.3 Image Capture and Analysis

The wicking kinetics images were captured using a Fujifilm XA1 camera.

3 RESULTS & DISCUSSION

3.1 Specimen Identification

Based on the tile test, it was confirmed that out of 27 samples tested, seven tubes were blood group A, four tubes were blood group B, and three tubes were blood group AB and 13 tubes were blood group O.

3.2 Determination Types of Blood Groups

All the paper strips treated with antisera A and antisera B were used to determine the types of blood groups. Based on Table 1 below, a drop of known blood group A was placed on the antisera A kit and the wicking distance observed spread till four (+) showing the presence of hemagglutination reaction due to the existence of antigen A on the surface of red blood cells. A reaction is considered a four (+) if the blood covered only the circle area in the middle of the paper strip. Secondly, a drop of known blood group B was placed on the antisera B kit and the wicking distance observed spread till four (+) demonstrating the occurrence of hemagglutination reaction due to the presence of antigen B on the surface of the RBCs. Thirdly, a drop of known blood group AB was placed on both antisera A and antisera B kits, and the wicking distance observed spread giving a three (+) reaction on both strips. A three (+) reaction is shown by having the blood spread not more than the first box besides the middle circle on the paper strips. This is because AB blood group contains both A and B antigen but the proportion of the antigens available are different than those appear in group A and group B red blood cells (Reid, M. E., & Mohandas, N., 2004). Subsequently, a drop of known blood group O was placed on each antiserum A and antisera B kits, the wicking distance gave a negative reactions on both strips. A negative reaction is shown through the spread of blood until the end of the strip, indicating that there is no antigen presence in the blood to react with the antibody that was coated on the paper strips.

TABLE 1. Results of antisera-A and antisera-B coated paper strips tested with known cells.

Type of blood group	Antisera-A	Antisera-B
A		
B		
AB		
O		

3.3 Blood separation and agglutination on paper

When the blood is deposited onto the paper, the blood will eventually moves by capillary action between the interfiber spaces in the paper (wicking). The reason behind this is the inequality in surface energy between the liquid and the solid, where the capability of the movement depends on the size of the interfiber spaces and the red blood cells. Friction and viscous dissipation are the resistances that will affect the rate of the movement and the distance of the separation (Khan *et al*, 2010). As such, the antibody coated paper strips can be used for blood typing where the comparison in wicking distance between a stable and a clumped RBCs specimen is used as the base of analysis. Blood agglutination can be induced by selective antigen that exists on the surface of erythrocytes and the relative antibody present on the treated paper strip.

CONCLUSION

In this preliminary study, all objectives have been accomplished successfully. Based on this experiment, it can be summarized that antibody coated paper strips have good potential to be used as an alternative for blood typing. Results obtained from the study shown a strong reaction towards the blood samples taken when tested with the antibody coated paper strips. However, several parameters need to be optimized for the paper to be produced as a kit in typing the blood group. The paper strips are cheaper, biocompatible and biodegradable compared to other materials for producing blood typing kits. As such, it will be beneficial for the health industry in manufacturing a cheaper kit while promoting health especially in developing countries.

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