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# STUDY ON MIXING ANTI-A MONOCLONAL ANTIBODY WITH ANTI-B MONOCLONAL ANTIBODY TO MAKE ANTI-AB USING FOR ABO BLOOD ISOTYPING

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**Abstract.** The ABO blood group system is divided into four different blood groups A, B, AB and O based on the presence of A and/or B antigens on the surface of human red blood cells. Blood type A is divided into subgroups, so AB blood type is also divided into subgroups depending on the subgroup A. The number of A or B antigen-receptors on red blood cells A, AB and B respectively are very different, depending on age and physiological state of the cells. However, in average, the relative proportion of A antigen receptors on red blood cells A versus the receptors on red blood cells AB is about 4:1. This ratio for B antigen sites on red blood cells B and AB is 2:1. The ratio of A antigen receptors versus B antigen receptor on red blood cells AB is approximately 3:2. Thus, the number of B antigen sites in all red blood types always appears with smaller amounts compared to the number of A antigen sites. Hence, in this study we focus to find a proper ratio of anti-A monoclonal antibody and anti-B monoclonal antibody in the mixing so that the possibility of agglutination will be the strongest. In this paper, anti-A monoclonal antibody (titer of 1/256) and anti-B monoclonal antibody (with titer of 1/256) were used. Three tests were carried out where each volume anti A monoclonal antibody was mixed with one, two and three volume anti-B monoclonal antibody separately. And the best results were obtained when one volume anti A monoclonal antibody was mixed with one volume anti-B monoclonal antibody. The corresponding titers of the anti-A,B antibody were 1/128 for red blood group A and 1/128 for red blood group B. The intensity of agglutination reached 3+ for both A and B red blood groups.

*Keywords:* anti-A monoclonal antibody, anti-B monoclonal antibody, anti-A,B monoclonal antibody, ABO blood group system, ABO blood grouping reagent.

*Classification numbers:* 2.7.1, 2.10.2.

## 1. INTRODUCTION

Human blood groups are usually classified based on the presence or absence of the antigen A and B, which are carried on the surface of the red blood cells. In 1901, Karl Landsteiner was the first person who recognized the existence of the ABO blood group system in humans, that group systems consists of A, B and O groups [1]. The AB blood group was named a year later

by Adriano Sturli and Alfred von Decastello – who were working under Landsteiner [2], when they discovered both of A and B antigen on the same red blood cell. This blood group was then further divided into subgroups. The most common subgroups of group A phenotype are A1 and A2 [3, 4, 5] and A3 [6]. The numbers of A antigen positions for the A1, A2, A1B, and A2B subgroups are about $(0.81\text{-}1.17)\times10^6$, $(0.24\text{-}0.29)\times10^6$, $(0.46\text{-}0.85)\times10^6$, and $0.12\times10^6$, respectively. The numbers of the B antigen positions for the B blood group and A1B subgroup are about $(0.61\text{-}0.83)\times10^6$ and $(0.31\text{-}0.56)\times10^6$, respectively [7].

Nowadays, anti AB blood grouping reagent has been commercialized by various companies. Anti-AB reagent of Biotest company contains monoclonal antibodies that belonged to IgM immunoglobulin class. Anti-A was produced by BS63 hybridoma cell line, and anti-B was produced by BS85 hybridoma cell line [8]. Anti-AB reagent of Diagast company is a mixture of 4 different monoclonal antibodies, of that two anti-A monoclonal antibodies were produced from 2521B8 and 16243G2 hybridoma cell line, and two anti-B monoclonal antibodies were produced from 16247E10 and 7821D9 hybridoma cell line [9].

It is a fact that up to now all serum samples using for the ABO blood type by serological methods in Vietnam have been imported. In 2016, Nguyen Thi Trung *et al.* successfully created the A6G11C9 hybridoma cell line that producing anti A monoclonal antibody, and the B4D10C9 hybridoma cell line that producing anti B monoclonal antibody, both of those monoclonal antibodies are isotyped IgM class [10]. A large amount of anti-A and anti-B monoclonal antibodies was produced. These monoclonal antibodies were used as materials for making ABO blood group reagent. Their antibodies titer was 1/256. In this paper, the anti-A and anti-B monoclonal antibodies were mixed with each other in order to make anti-A,B reagent. Among these three tests carried out where each volume anti A monoclonal antibody was mixed with one, two and three volumes anti-B monoclonal antibody separately to find the best intensity of agglutination and the anti-A,B antibody titer for both red blood group A and B.

## 2. MATERIALS AND METHODS

### 2.1. Materials

#### *2.1.1 Red blood cell samples*

National Institute of Hematology and Blood Transfusion, Viet Nam provided a set of three blood types: red blood cells A sample (5 %), red blood cells B sample (5 %), red blood cells O sample (5 %) for this study.

#### *2.1.2 Monoclonal antibodies*

Anti-A monoclonal antibody (titer of 1/256), and Anti-B monoclonal antibody (titer of 1/256) produced by Project code KC04.13/11-15 were used to combine into anti-A,B reagent.

#### *2.1.3 Serum samples*

A set of anti-A, anti-B and anti-AB were purchased from Bio-Rad (France), and used as positive controls.

### 2.2. Analytical methods

*2.2.1. Method for making anti-A,B antibodies*

Anti-A, anti-B monoclonal antibodies were prepared by dilution a tock solution in PBS pH7.8 containing 22 % BSA, 0,01 % sodium azide, 10 mM EDTA, to reach the antibody titer of 1/256. Then the antibodies were mixed together in the ratio 1A:1B, 1A:2B, 1A:3B. The specificity agglutination of antibody to red blood cells, antibody titer and the intensity of antibody was investigated.

*2.2.2. Methods for red blood cells agglutination*

Red blood cell agglutination was performed in the U/V bottom wells of plastic plate as the following: transferred 25 µl of the cultured medium/serum samples/antibody in each well of 96 wells plastic plate; added 25 µl sample red blood cells (1 or 2 %); slightly mixed and placed at the room temperature for 30 minutes; tilted at a 45 degree angle to read results.

Red blood cell agglutination on the slide/ceramic: Marked 3 positions on the slide, each position was spaced others about 5-6 cm; dropped in each position 25 µl of red blood cell sample, in the order form: position 1 is red blood cell group A, position 2 is red blood cell group B and position 3 is red blood cell group O; added 25 µl of the cultured medium/serum samples/antibody into the positions of red blood cell above; mixed them by the glass rods and then gently shaked the slide during 3 minutes; read and recorded results.

*2.2.3. Methods for determining the specificity of antibody*

The specificity of the antibody (of cultured medium) was determined by the agglutination of red blood cell groups A, B and O with procedures corresponding to the provisions of Circular No. 26/2013-TT-BYT dated on 29/3/2013. Accordingly, the specificity of the antibody was determined by the erythrocytes agglutination on the slide.

*2.2.4. Method for determining the titer of antibody*

An antibody titer is the greatest dilution (in a serial dilution) that still gives a positive result. So, a series of serum dilutions of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512 was prepared then transferred 25 µl of each dilution in plate wells. The wells were supplemented with 25 µl of 2 % red blood cells; mixed well and leaved at room temperature for 30 minutes; observed the results by tilted the plate at a 45 degree angle. The positive (antibodies from Bio-Rad) and negative (DMEM medium containing 10 % FBS (WHO, 2006)) controls were performed in parallel.

*2.2.5. Methods for determining the intensity of antibody*

Dropped 25 µl of culture fluid onto a clean glass plate's surface; added an equal volume of a 10 % red blood sample; mixed them with a glass rod then observed agglutination.

Depending on the titer of the serum antibodies, agglutination occurred instantly or within seconds. Used a stopwatch to determine the time and observed the first signals of agglutination. Five minutes later, the intensity of the reaction antigen – antibody was assessed. The intensity was indicated by values from 1+ to 4+ [11].

## 3. RESULTS AND DISCUSSION

### 3.1. The intensity of individual antibodies

Before mixing, the intensity and specificity of anti-A and anti-B monoclonal antibodies were tested. Figure 1 showed that the intensity of both monoclonal antibodies was 4+, that was presented by agglutination.

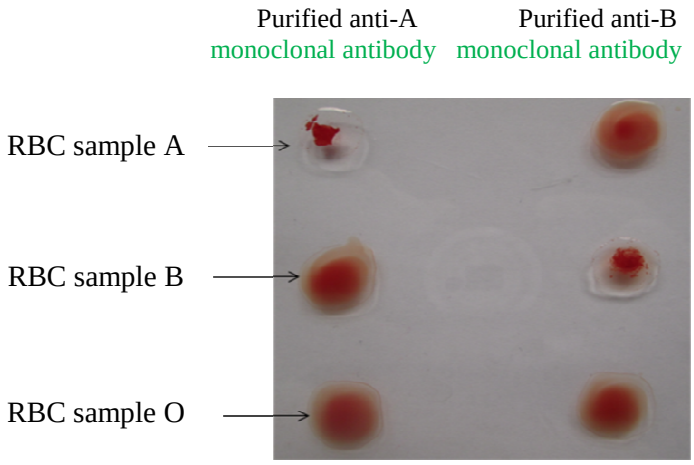


*Figure 1*. The intensity of anti-A, anti-B monoclonal antibodies.

### 3.2. The ratio of two antibodies

The anti-A monoclonal antibody and the anti-B monoclonal antibody were mixed with each other in various proportions. The intensity of the each mixture antibodies was test (Figure 2).

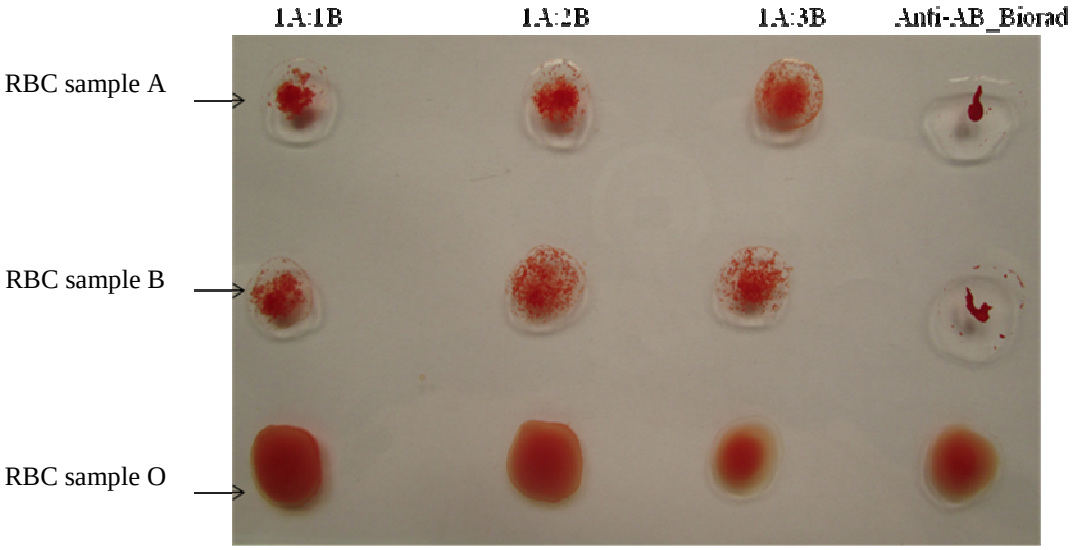


*Figure 2*. The intensity of the mixed anti-A/anti-B in some proportions.

The results in Figure 2 showed that the mixed anti-A/anti-B in some proportions caused hemagglutination of the red blood cells carrying antigens A and red blood cells carrying antigens B. The serum sample anti-AB (Bio-Rad) caused strongly agglutination for both red blood cells carrying antigens A and red blood cells carrying antigens B, equivalent to 4+ intensity. Meanwhile, the intensity of the mixed anti-A/anti-B in all three ratios were only about 3+ due to existence of small clusters of cells in the liquid surrounding the agglutination position. For the case with the ratio of anti-A/anti-B was 1:1 (v/v), the number of the clusters of cells was smaller, but the size of the agglutination was larger compared to the other 2 cases. It means the intensity of this mixed antibodies was better than that of two remaining positions (Figure 2), or in other words, the best results achieved with the mixing ratio of two antibodies was 1v: 1v (anti-A: anti-B). According to [7], the ratio of A antigen sites and B antigen sites per red blood cell AB is about 1.5. So, the results of this research are in agreement with the Economidou' results.

### 3.3. Make anti-A,B as serum sample

As mentioned above, the anti-A,B was produced by mixing anti-A monoclonal antibody with anti-B monoclonal antibody. The anti-A,B product was used to diagnose AB blood group. Theoretically, in order to distinguish four ABO blood types, only two kinds of serum samples as anti-A and anti-B are enough, but in practice, three kinds of serum samples are using. The use of ABO blood grouping reagent containing anti-AB has increased the reliability of the results.

Figure 3 and 4 show that the anti-A,B product created by the present study was as good as the product of Bio-Rad. The intensity of anti-A,B was 3+ for both red blood cell A and red blood cell B. The titer of anti-A,B product created by the present study with red blood cell A and red blood cell B reached 1/128.

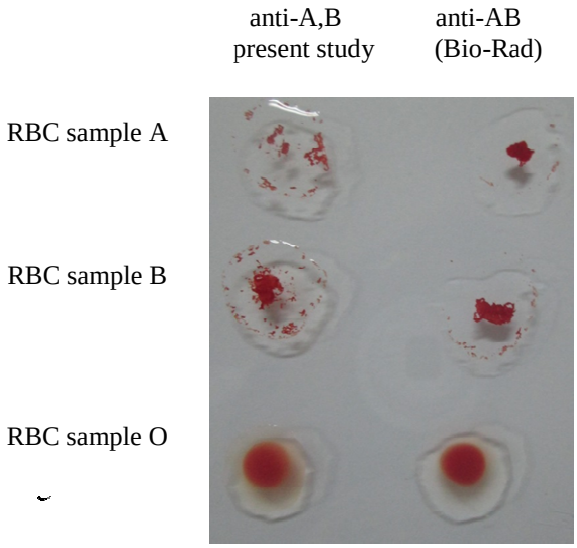


*Figure 3.* Hemagglutination reaction of anti-A,B product created by the present study.

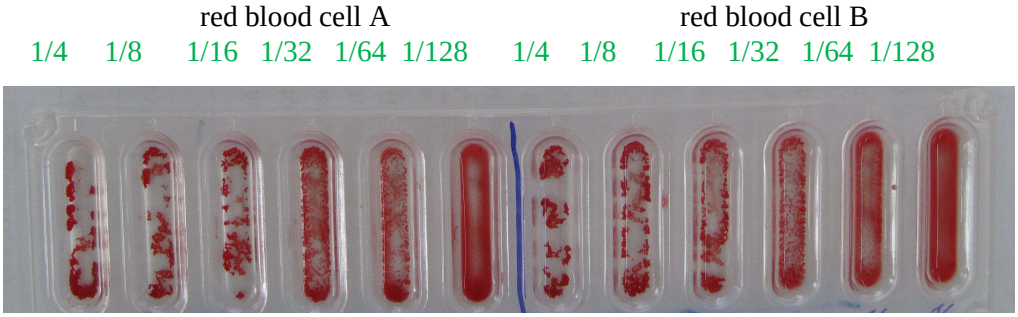


*Figure 4.* The titer of anti-A,B product created by the present study with red blood cell A and red blood cell B.

## 4. CONCLUSIONS

Main results of the present study can be summarized as follows: The mixing ratio of the anti-A monoclonal antibody (titer 1/256) with the anti-B monoclonal antibody (titer 1/256) is 1:1 in volume. The intensity of anti-A,B product is 3+ for both red blood cell A and red blood cell B. The anti-A,B product has antibody titer is 1/128 with both red blood cell A and red blood cell B.

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