Optimization Concentration Control Cell Coombs (CCC) for Validity Tests on Crossmatching Examination

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Abstract. Crossmatching is an examination that needs to be done before doing a blood transfusion to see whether the recipient's blood is in accordance with the donor's blood. Validity is done to find out the results obtained in all phases crossmatching tests correctly indicate compatible. Control Coombs Cell (CCC) is a control cell suspension made from group O Rh positive blood which is intentionally coated with an incomplete antibody. The aim is to determine the concentration and optimal time of CCC incubation so that it can be used for validity testing on crossmatching examination. The test used two-way ANOVA test showed significant results, there were differences in the degree of agglutination in variations in concentration and incubation time. The optimum results were not obtained at concentrations of 50 percents, 60 percents, 70 percents and the right incubation time of 15 minutes, 30 minutes, and 60 minutes. The results of agglutination were reduced at a concentration of 50 percents and an incubation time of 15 minutes, that coombs as controlling negative CCC test results and testing serum coombs. CCC as validating results so as not to cause false positive reactions or false negative results.

Keywords: Crossmatching, Validity, Control Cell Coombs (CCC), incubation time, concentration

1. Introduction
Crossmatching examination is needed before blood transfusion to see whether the recipient's blood matches the donor's blood. Crossmatching examination is very necessary so that recipients get a suitable blood transfusion without any danger. The transfusion reaction that occurs is caused by giving blood that is not compatible with the recipient, to minimize the occurrence of transfusion reactions, it is imperative to do a crossmatching test which is the last gate to find out whether the donor blood will be transfused to compatible recipients so that crossmatching is the last indicator to decide whether blood products should be given or transfused to recipients. Although the blood groups of ABO and Rhesus are both recipient and donor known, crossmatching examination must be carried out as thoroughly and as well as possible in order to minimize errors so that antibodies that can cause transfusion reactions can be detected and so that the transfused blood does not cause a suitable transfusion reaction without danger [1].

Crossmatching examination is shown to determine the match between patient or recipient blood and donor blood before blood transfusion. In general, crossmatching is divided into two, namely: Cross Match Major is intended to detect the presence of antibodies in recipient serum that can destroy donor red blood cells and Cross Match minor is intended to detect the presence of antibodies in donor serum that can destroy recipient red blood cells [2].
The main purpose of crossmatching is to prevent transfusion reactions, both life-threatening transfusion reactions and mild or moderate transfusion reactions that can interfere with patient comfort. Another important goal is to maximize the life span of in vivo transfused red blood cells [3]. If agglutination or hemolysis is positive the results of crossmatching are declared incompatible. If agglutination or hemolysis is negative in all tubes, the results are negative or compatible and proceed with the addition of CCC by 1 drop and followed by centrifugation for 1 minute at a speed of 1000 rpm. Addition of CCC will give positive results on all negative results that show valid examination results. If with the addition of CCC the reaction remains negative, then the examination is declared invalid and must be repeated [4].

Validity Test is done to find out the results obtained in phase I to III crossmatching tests correctly show that they are compatible. Validity test is done by adding CCC as much as 1 drop into the tube that the coombs test results are negative in phase III. CCC is a control cell suspension made from blood type O Rh (+) which is intentionally coated with an incomplete antibody. The use of CCC aims to determine whether coomb's serum used in phase III is still active or not, if it is still active the addition of CCC into Coomb's serum gives a positive reaction (agglutination). After that, the tube containing the mixture was centrifuged for 15 seconds at 3000 rpm. The reaction is read against macroscopic and microscopic hemolysis and agglutination. But in this practicum only macroscopic observations are made. From this validity test results were obtained, the major showed a positive reaction 1 (small lumps with red liquid around it), minor, auto control and auto pool also showed a positive reaction 1 (agglutination with small lumps and red liquid). These results indicate that the crossmatching test was declared valid. Positive results on the validity test and negative (compatible) results of the three phases indicate that blood from donors is safe to give or transfuse to patients [5].

Based on the preliminary test that the researchers did, the Anti-D variation of 50% 60% 70% was used, yielding positive results but before the validation test had already produced positive results. Based on the background above, the authors conducted a study on the check optimization of CCC concentration to test the validity of the crossmatching examination.

2. Methods
In this study requires a red blood cell suspension. The purpose of making cell suspensions is to optimize the antigen-antibody reaction so that the reactions that emerge can be clearly observed. Some literature states that 3% cell suspension is widely used for serological examination. However, according to the World Health Organization (WHO), 5% cell suspension is commonly used for serological procedures [4].

This study is an experimental study [6], meaning that in this study variations in the addition of anti-D and erythrocyte volumes of 5% were used to validate the Blood Tube method in Cross Match of 3 mL for donor erythrocyte and plasma blood type O patients.

2.1. Preparation of Plasma
1. 3 mL of blood are put into a tube that contains 30 µL Na.EDTA and labeled according to the sample.
2. The sample is closed by using parafilm.
3. The sample tube is rotated in a centrifuge with a speed of 3000 rpm for 15 minutes.
4. Plasma separated from red blood cells is separated into aliquot tubes which are then labeled according to the sample [7].

2.2. Preparation of Erythrocyte 5%
1. Prepare the tools and materials used.
2. 3 mL venous blood is inserted into a tube containing 30 µL 10% Sodium EDTA [7], then covered with parafilm, then homogeneous.
3. Centrifuge for 15 minutes at 3000 rpm.
4. Plasma and blood cells are separated and labeled.
5. Red blood cells that have been separated from plasma add 0.85% physiological NaCl, then cover with parafilm, then homogenize.
6. Centrifuge with a speed of 3000 rpm for 1 minute.
7. The supernatant formed is removed (upper liquid) and added 0.85% NaCl again. Do this for 3 times.
8. After the 0.85% NaCl fluid is removed, the remaining red blood cells are 100% erythrocyte suspension.
9. Take 1 part cell 100% + 19 parts NaCl 0.85% = 5% erythrocyte suspension [4].

2.3. Preparation of CCC
1. Mix 1 drop of Anti D (IgG) + 39 drops of saline, to obtain anti D dilution of 1/50, 1/60, 1/70.
2. In another tube mix 1 drop of washed erythrocytes in the positive O Rh group + 19 drops of saline so that a 5% suspension is obtained.
3. Mixed with anti D 1/50, 1/60, 1/70 + suspense washed O Rh positive 5% 5:20.
4. Incubation 15,30,60 minutes at 37°C.
5. Wash with saline 3 times (centrifuge 1500-2000) rpm for 3-5 minutes.
6. Discard 5% suspense from the solution again [8].

2.4. Indirect Coombs Test [9]
1. Prepare serum from donor or patient blood sample (after blood type and rhesus examination).
2. Prepare 5% erythrocyte suspense in saline from a donor or patient blood sample (1 part erythrocyte 100% + 19 parts NaCl 0.85% = erythrocyte suspension 5% = 1/20).
3. Mix 20:5 (20 drops of erythrocyte 5%: 5 anti-D drops) from each of the above suspicious erythrocytes with the serum to be examined according to their class (for Major and Minor).
4. Wash with saline solution 3 times. Then add to each 2 drops of Serum Coombs (Anti Human Globulin).
5. Centrifuge with a speed of 1000 rpm for 1 minute. Read the reactions that will occur macroscopically and microscopically.

2.5. Validity Test [4]
1. An empty tube is added 1 drop of CCC to test the Serum Coombs (Anti Human Globulin).
2. Re-centrifuged at 3000 rpm for 1 minute.
3. If there is no reaction the examination must be repeated (indicating that the Coombs serum is already bad). If there is a reaction then the blood donor is suitable given to the patient (indicating that the Coombs serum is still good).

3. Results and Discussion
Research has been conducted on the differences in concentration and time in the manufacture of CCC on the degree of agglutination. Information on the assessment criteria for the gradation of CCC agglutination [4]:

- Agglutination (±): Extremely fine lumps of red turbid supernatant
- Agglutination (+1): Large lumps, supernatant appear turbid
- Agglutination (+2): More lumps and rough, slightly clear fluid
- Agglutination (+3): Sediment splits into clear, clear fluid
- Agglutination (+4): All sediments unite, clear liquid

Research has been conducted on the making of CCC on the influence of concentrations of 50% (Fig. 1, 2), 60% (Fig. 2, 3), 70% (Fig. 4) and incubation time of 15 minutes, 30 minutes, 60 minutes.
Figure 1. Anti D 1/49 + Erythrocyte 5% (a) 15 minutes (+); (b) 30 minutes (+1)

Figure 2. (a) Anti D 1/49 + Erythrocyte 5% 60 minutes (+2); (b) Anti D 1/59 + Erythrocyte 5% 15 minutes (+2);

Figure 3. Anti D 1/59 + Erythrocyte 5% (a) 30 minutes (+1); (b) 60 minutes (+2)
Test the validity of a reagent is a test step carried out on the content of a reagent, with the aim to measure the accuracy of the reagent used in an examination. To find out whether the test is valid or not must be done through testing of the reagent itself so that the results of the examination can take place properly and correctly. By conducting a reagent validity test it is also useful to know the condition of the reagent [10].

Validity Test is to find out the results obtained in phase I to III crossmatching tests correctly show that they are compatible. Validity test is done by adding 1 drop of CCC into a tube which results in negative coombs test in phase III. CCC is a control cell suspension made from O Rh (+) blood group which is intentionally coated with an antibody incomplete. The use of CCC aims to find out whether coomb's serum used in phase III is still active or not, if it is still active the addition of CCC into Coomb's serum gives positive reaction results/agglutination [11].

CCC is a positive rhesus O blood cell suspense that has been sensitized (coated) by anti-D IgG (incomplete). The purpose of making CCC include testing whether Coomb's serum reagent is still valid or not and can test negative results from crossmatching tests, Direct Coomb's Test, and Indirect Coomb's Test [8].

In the research conducted is an experiment in making CCC where the making of this reagents is as a control result of a negative CCC test and testing coombs serum CCC as validating the results so that no positive false reactions or false negative results occur. The anti D IgG (monoclonal) that used in this which is only an incomplete antibody and has a high enough IgG titer to find out the anti D incomplete titer by obtaining an ideal CCC against anti D. This titration must be done by double/serial dilute henceforth obtained ascertained value of anti D reaction dilution which will be used in making CCC. In this study an indirect coombs test was examined using donor serum tested with recipient erythrocytes [4].

The CCC was made to be added in the validity test, firstly making a 100% erythrocyte suspense then made a 5% erythrocyte suspension [12]. However, in this study, variations in the concentration and incubation time of CCC were made, the concentration used was 50%, 60%, 70% on the incubation time variations used were 15 minutes 30 minutes, and 60 minutes.

In previous studies conducted using a concentration of 40% and 30 minutes, erythrocytes 5% and Anti-D used was equivalent to washing as much as 3 times, after that the results of agglutination were large and very visible. In this study, negative agglutination was not found in the CCC at a concentration of 50% (Fig. 1, 2), 60% (Fig. 2, 3), 70% (Fig. 4) and the incubation time was 15 minutes, 30 minutes, 60 minutes, but the results of agglutination were reduced from before, at a concentration of 50% and
time 15 minutes incubation obtained the smallest agglutination results, and has been seen on a 50% concentration microscope and 15 minutes can be used in the addition of CCC in the validity test to determine the workmanship in phase I to phase III of the reagents used are still active or not. Proven by macroscopic examination seen in microscopically there is a separate erythrocyte and CCC can be used. Furthermore, the data analysis was performed statistic test, 2-way ANOVA. Based on the concentration variation, has a sig value <0.05; 0,000 <0.05; there are differences in the degree of agglutination in variations in concentration. If incubation variations, have a sig value <0.05; 0.000 <0.05, there is a difference in the degree of agglutination in the incubation time variation. And the relationship between the results of the variation of concentration with incubation time, has a sig <0.05; 0.005 <0.05; there are differences in the degree of agglutination in the variation of concentration against the incubation time variation. Conducted in the experiment of making CCC aims to control the results of the negative test and test the serum coombs. CCC as validating the results so that there are no false positive reactions or false negative results.

4. Conclusion
From this study, no optimum results were obtained for concentrations of 50%, 60%, 70% and the correct incubation time of 15 minutes, 30 minutes, 60 minutes, but the results of agglutination were reduced at 50% concentration and 15 minutes incubation time, that coombs control cell as controlling the results of the negative coombs control cell test and testing the serum coombs, coombs control cell as validating the results so that no false positive reactions or false negative results occur.

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