ANALYSIS OF THE RESULTS OF THE ABO BLOOD BLOOD CLASS EXAMINATION WITH ANTISERA REAGENT IS ENROLLMENT AND NOT DECLARATED AT THE OTANAHA GORONTALO Hospital

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ABSTRACT

Blood is part of the body's transport system which consists of two parts, namely blood plasma and blood cells consisting of erythrocytes, leukocytes, and platelets. One of the blood tests is the determination of the type of blood by looking at the degree of agglutination. The effect of diluting the antisera reagent on agglutination is not yet known. This study aims to see the differences in the results of agglutination in the ABO system blood group examination with diluted and undiluted antisera reagents.

This type of research is quantitative research with a quasy experimental design. The sampling technique was purposive sampling with a total sample size of 20 experimental respondents (50%, 40%, and 20% Antisera dilution) and control (without dilution) and then compared the experimental and control results using the comparative non-parametric Kruskall-Wallis test. ($\alpha = 5\%$).

The results of this study are that there is a significant difference (0.00 <0.05) between the experimental and control groups (0.00 <0.05) and there is a significant effect (H hit> H table) between the experimental and control groups (151,674> 77.930) So it can be concluded that there is a significant difference and influence on the analysis of the results of the ABO system blood group examination agglutination with diluted and undiluted antisera reagents. The decrease in the degree of agglutination from the results of the blood group examination is caused by the higher dilution, which affects the antibody ratio in the antisera.

Keywords : Agglutination, antibody ratio, dilution

PRELIMINARY

Blood is a fluid composed of two parts, namely plasma and blood cells. Blood plays an important role in maintaining the balance of the body and as a medium or transportation of various materials from one cell to another. The function of blood is to buffer changes in pH, transport excess heat out of the body, change the body's system, and minimize blood loss when there is damage to blood vessels through hemoestasis [15].

The minimum blood pre-transfusion test that must be done in the laboratory is examination of the ABO and rhesus system blood groups and cross-matching [22].

Establishment of blood transfusion service standards becomes a reference for health workers and program implementers in the health sector in providing blood transfusion services. Blood transfusion service standards aim to ensure safe blood services and adequate quantities [3].

Blood type is very important to know for transfusion purposes. The right donor and is also often used in cases of forensic medicine such as identification DNA examinations and laboratory tests. In laboratory examinations, there are several types of examinations performed, one of which is the type of immunohematological examination which is a branch of hematology that studies antigen and antibody reactions as well as analogue phenomena related to the pathogenesis and clinical manifestations of blood disorders. Most of the techniques used in UTD laboratories (Blood Transfusion Units) and blood banks to detect reactions between antigens and antibodies are based on agglutination techniques.

In general, blood has 4 groups, namely: blood group A, where blood group A has antigen A and antibody B, blood group B where this blood group has antigen B and antibody A, blood group AB where this blood group has antigen A and B but does not have antibodies, and blood type O is the blood type that does not have antigens but has antibodies A and B. The term blood type is certainly familiar among the public, but it cannot be denied that there are still many people who do not know their blood type, including people who are in rural areas or with low economic status. Actually, not a few of them know the importance of blood type, but the means and costs are sometimes the main reasons.

Various urgent interests related to the above include as a reference to the necessary requirements, namely as a donor or recipient. Another thing is in the form of identity management requirements, including for Identity Cards, Driving Permits, and also as genetic markers [11].

ABO blood group examination is performed to determine the type of blood group in humans. ABO blood group determination is generally carried out using the slide method. This method is based on the principle of reaction between agglutinogens (antigens) on the surface of erythrocytes with agglutinins present in serum / plasma which form agglutination or clots. The slide method is a simple, fast and easy method for blood group examination [16].

In the blood group examination, the reagent used was antisera, which is a commercially available reagent which is also in practice used in a ready-to-use form. Currently, several clinical laboratories that are running health programs, both from the Puskesmas, Clinics, Hospitals, and other agencies have diluted blood type reagents with physiological NaCl against ready-to-use antisera. Until now, how the effect of the diluted antisera on the results of ABO blood group testing with the ratio between the reagent that was diluted and the

reagent without dilution is still not clearly known [11].

Based on the research entitled "The Effect of Antisera Dilution Variations on the Results of Landsteiner ABO Blood Type Examination" this study aims to determine and determine the effect of antisera dilution variations on the results of ABO blood group examination using 1: 1, 1: 2, 1: 3, and 1 dilution variations. : 4 it has been found that there is a significant effect on the results of the examination. Furthermore, the researcher wanted to know the effect of using antisera dilution on the results of the ABO system blood group examination [11].

Based on the above background, the researcher is interested in conducting a study entitled "Analysis of Blood Type Agglutination Results Using Diluted and Undiluted Antisera Reagents at the Otanaha Regional General Hospital, Gorontalo".

RESEARCH METHODS

This type of research is experimental research with a quantitative approach, namely the type of research that aims to conduct research with treatment, control use, and laboratory repetition or duplication for the analysis of the results of the ABO system blood group examination with antisera reagents, Diluted and Undiluted in Regional General Hospitals Otanaha Gorontalo City.

The location of the research was conducted at the General Hospital Otanaha Gorontalo. The population in this study were all 40 outpatients at the Regional General Hospital of Otanaha Kota Gorontalo in 2020. The sample size of 20 samples was obtained from the formula for estimating proportions with known populations.

The results of the examination obtained are primary data, which are then made in the form of tables using univariate data analysis techniques by describing or describing variables then followed by bivariate data analysis techniques to test comparative hypotheses if there are (differences or comparisons) in the analysis of agglutination results in the blood group examination system. ABO with diluted and undiluted antisera reagent.

The variables in this study use independent variables (independent), namely variables that affect the results of the study, and dependent variables (dependent), namely variables that are influenced by the independent variables. What acts as the independent variable in this study is the antisera volume of ABO blood group with dilution (50%, 40%, and 20%), while the dependent variable in this study is the agglutination result of blood group examination with ABO system.

The instrument in this research is the tool used in this research is Torniquet, 3ml syringe, cotton, test tube, tube rack, micropipette, yellow tip, glass slide, rotator, magnifying glass, and microscope. The materials used were EDTA blood, 70% alcohol swab, anti-scab reagent (Anti A, anti-B, and anti-AB), 0.9% NaCl pH 6-7, and pH paper. Then the antisera reagent was diluted with dilution (50% dilution, 40% dilution, and 20% dilution). Labeled each tube and kept it at 2-8 ° C. Store the reagent at room temperature before use at 2 ° C-8 ° C., Separate the serum and plasma from red blood cells, then wash the red blood cells to make cell suspensions, Label each well, i.e. well 1 is labeled -A , Well 2 is labeled -B, Well 3 is labeled EA, Well 4 is labeled EB, Well 5 is labeled EO, and Well 6 is labeled AK,

Drop each cell 1 drop of the patient's 10% suspension of red blood cells in well 1,2,6, Drop each of 2 drops of patient serum / plasma into well 3,4,5,6 Mix by shaking the bioplate back and forth until well blended ± 2 minutes then read the reaction.

The sampling technique in this study is purposive sampling, which is where the research sample is selected according to the criteria that the researcher has set. The inclusion criteria in the study: Respondents were willing to be the sample of the study, namely blood group examination, Respondents did not have a history of AIHA disease. Exclusion criteria in the study: Lysed EDTA blood.

Operational definition in this research:

Antisera Dilution; The concentration of antisera is the difference in the volume of the antisera with the addition of physiological NaCl to obtain antisera dilution (50%, 40%, and 20%).

Agglutination; It is adhesion of red blood cells caused by antibodies (antisera) that attach to the antigens of some red blood cells, causing a webbing that can ensnare the cells into clusters.

RESEARCH RESULT

Table 1. *Results of Blood Type Examination Research*

Research Samples	Blood Type	Treatment	Dilution			
			Control	50%	40%	20%
R1 Ny. A.S	Golda A	1	4	4	4	3
		2	4	4	4	3
		3	4	4	3	2
R2 Tn. R.U	Golda A	1	4	3	3	2
		2	4	3	3	2
		3	4	3	3	2
R3 Tn. N.B	Golda A	1	4	4	4	3
		2	4	4	4	3
		3	4	4	3	2
R4 Tn. R.T	Golda A	1	4	3	3	2
		2	4	3	3	2
		3	4	3	3	2
R5 Ny. A.D	Golda A	1	4	4	4	3
		2	4	4	4	3
		3	4	4	3	2
R6 Tn. T.U	Golda A	1	4	3	3	2
		2	4	3	3	2
		3	4	3	3	2
R7 Ny. K.D	Golda A	1	4	3	3	2
		2	4	3	3	2
		3	4	3	3	2
R8 Ny. L.A	Golda A	1	4	4	4	3
		2	4	4	4	3
		3	4	4	3	2
R9 Ny. M.W	Golda B	1	4	4	4	3
		2	4	4	4	3
		3	4	4	4	3
R10 Ny. S.W	Golda B	1	4	3	3	2
		2	4	3	3	2
		3	4	3	3	2
R11 Tn. J.S	Golda B	1	4	4	3	3
		2	4	4	3	3
		3	4	3	2	2
R12 Ny. N.I	Golda B	1	4	3	2	2
		2	4	3	3	2
		3	4	3	3	2
R13 Ny. H.D	Golda B	1	4	4	3	3
		2	4	4	4	3
		3	4	4	4	3

R9 Ny. M.W	Golda B	1	4	4	4	3
		2	4	4	4	3
		3	4	4	4	3
R10 Ny. S.W	Golda B	1	4	3	3	2
		2	4	3	3	2
		3	4	3	3	2
R11 Tn. J.S	Golda B	1	4	4	3	3
		2	4	4	3	3
		3	4	3	2	2
R12 Ny. N.I	Golda B	1	4	3	2	2
		2	4	3	3	2
		3	4	3	3	2
R13 Ny. H.D	Golda B	1	4	4	3	3
		2	4	4	4	3
		3	4	4	4	3

Source: Data processed in 2020

Based on table 4.1, the total number of research samples is 240 samples with the experimental group totaling 20 respondents (R1 to R20) and the control group totaling 20 respondents (R1 to R20). The results of the blood group examination were treated 3 times for each group (Control), and dilution (50%, 40%, and 20%). The data above shows that the higher the dilution, the weaker the agglutination rate will be.

Table 2. *Distribution of Blood Type Examination Frequency*

Blood Type	Frequency
A blood type	8
Blood Type B	6
Blood Type AB	6
Total	20

Source: Data processed in 2020

Frequency distribution is a list, table, or diagram that shows the frequency of events in a sample [5]. Based on table 4.2 above, it can be seen that there were 8 patients with blood type A, 6 patients with type B blood, and 6 patients with AB blood type who were undergoing outpatient treatment at the General Hospital of the Otanaha Region, Gorontalo.

Table 3. *Normality Test of Blood Type Examination Results*

Check Up Results	Antisera Dilution	Significant	Significant Level	Information
Eksperimen	50%	0,00	0,05	Tidak Normal
	40%	0,00	0,05	Tidak Normal
	20%	0,00	0,05	Tidak Normal
Kontrol	Tanpa Pengenceran	0.00	0,05	Tidak Normal

Source: Data processed in 2020

In the Shapiro Wilk test, the data is said to be normally distributed if the significant value is> the significance level (5% or 0.05) [17]. Based on Table 4. The results of the normality test analysis of the ABO system blood group examination with antisera reagents are diluted and not diluted in the hospital. Otanaha Gorontalo obtained the results of the experimental group, namely the dilution of 50%, 40%, and 20% was 0.00 <0.05. So, it can be seen that the data obtained are not normally distributed. In addition, the normality test results "Normality test results for ABO System Blood Group Agglutination Results Analysis with Antisera reagent Diluted and Undiluted at Otanaha Regional General Hospital, Gorontalo" in the control group, namely without dilution, was 0.00 <0.05. Then, The data obtained is also data that is not normally distributed. Referring to this, it can be concluded that the data obtained are all not normally distributed so that it can be continued on the Non-Parametric comparative analysis test, namely the Kruskall-Wall Test.

Table 4.
Results of the Kruskal Wallis Test Analysis

Chi-Square	Check up result
	151,674
Df	3
Asymp. Sig.	.000

Source: Data processed in 2020

From the table above shows If the value is Asymp. Sig <0.05. Then there is a difference or Ho is rejected and Ha is accepted, meaning that there is a difference between each group (Control) and the experimental group (50%, 40%, 20% dilution).

DISCUSSION

[1] Blood is an important part of the transport system and is a fluid-shaped tissue consisting of two parts, namely

blood plasma and the corpuscles which consist of leukocytes, erythrocytes and platelets. In medicine, it is known that the division of blood groups into four blood groups, namely blood groups A, B, AB, and O. This division is done because of the different types of carbohydrates from proteins on the surface of the red blood cell membrane. To find out a person's blood type, laboratory tests are needed. So far, in conducting blood type checks, the ABO system is often used which is done manually or by dropping three types of fluids or reagents on the examination sample.

Blood type is a special characteristic of red blood cells which have different protein and carbohydrate contents. Information about the type of blood group is very important to know, especially in the blood transfusion process. This is due to avoiding immunological reactions due to differences in the chemical composition of erythrocytes between recipients and donors. In the ABO blood group system is based on agglutination between antigens on normal red blood cells (agglutinogens) and antibodies in normal individual serum (agglutinins). The antigen on red blood cells is in the form of antigen A and antigen B. Individuals with blood group A have antigen A on their red blood cells and anti-B antibodies, individuals with blood group B have antigen B and anti-A antibodies, individuals with blood group AB are present AB antigen and has no antibodies,

The agglutination technique that is often used in UTD laboratories and blood banks to detect antigen and antibody reactions is based on agglutination techniques. Agglutination in blood group examination is the adhesion of red blood cells caused by antibodies that attach to the antigens of some red blood cells, causing a webbing that can entangle the cells into clusters. Agglutination of red blood cells in blood group examination can take place in two stages. The first stage the antibody binds to the surface of the red blood cell, the second stage the antibody interacts with the red blood cell so that the cells are close together and agglutination occurs.

The first stage of agglutination is influenced by temperature, medium pH, antibody affinity constant, incubation time, ionic strength in the medium, and the ratio of antigen and antibody. The second stage of agglutination is influenced by the distance between cells, the molecular charge of the membrane surface and the molecular structure [10].

The results of agglutination examination of the ABO system blood group with antisera reagent diluted and not diluted in blood group A using antisera A, blood group B using antisera B, blood group AB using antisera AB. The experimental group in the form of a dilution (50%, 40%, 20%), and the control group (without dilution) after the examination and seeing the degree of agglutination showed a strong agglutination reaction in the control (without dilution) compared to the experimental group antisera in the form of dilution (50 %, 40%, and 20%) which were diluted using physiological NaCl. This is influenced by the number of antibodies in the antisera A, B, and AB in the control group more than the number of antibodies in the antisera A, B, and AB in the experimental group in the form of dilutions (50%, 40%,

The dilution of antisera was carried out by dilution experimental group (50%, 40%, and 20%) and control group (without dilution). Based on the research (Naim, 2015) dilution variations of 1: 1, 1: 2, 1: 3, and 1: 4 have been carried out and blood samples A, B, and AB have a significant effect so that dilution is carried out but with a higher concentration level. dilution (50%, 40%,

and 20%) to see the extent of the effect resulting from the dilution [11]

In the experimental group, 50% dilution was made for each antisera A with a concentration of 50%, antisera B with a concentration of 50%, and antisera AB with a concentration of 50% of the 100% concentration or without dilution by calculating the volume to be made 2 ml, the volume required in the dilution of the antisera is 1 ml using physiological NaCl as a diluent up to the 2 ml limit mark to obtain the desired concentration. In the experimental group with a 40% dilution, each antisera A with a concentration of 40%, antisera B with a concentration of 40%, and antisera AB with a concentration of 40% of the concentration of 100% or without dilution with the calculation of the volume to be made 2 ml, the volume required in the dilution of the antisera is 0, 8 ml using Physiological NaCl as a diluent to the 2 ml limit mark so that the desired concentration is obtained. In the 20% dilution experimental group, 20% concentration of antisera A, 20% concentration of antisera B, and 20% concentration of AB antisera from 100% concentration or without dilution was made by calculating the volume to be made 2 ml, the volume required in the dilution The antisera was 0.4 ml using Physiological NaCl as a diluent to the 2 ml limit mark so that the desired concentration was obtained.

The results of agglutination examination of the blood group with a 50% dilution can be seen the results of the degree of agglutination (+4), meaning that it still gives the same results as the control group but the time or duration of agglutination is slightly longer, this is influenced by the level of dilution which reduces the number of antibodies that are in antisera so that the agglutination formation process takes a little longer than the reading of the results. In the respondent (sample) number 2,4,6,7,10 there is a difference in the results of the degree of agglutination (+3) this is due to the fast reading time of the respondent (sample) number 1,3,5,8,10. The same thing was seen with antiseraB and AB which also had a result (+3) but there were more AB antisera in 5 samples compared to antisera B with 3 samples. This difference is caused because each blood group of each individual has different antigenic strength, while for the difference in the reading of the results on the same antisera and respondent (sample) but different reading time or still within $\leq$ 2 minutes. At 40% dilution, it can be seen that the sample respondents 11,16,17, and 19 experienced a decrease in the degree of agglutination again to (+2) (Macroscopic observation), this is because the time and readings were a little longer than the previous work that should have been Immediately note the results of the reaction, because with the examination of many samples and the reading of the reaction must be faster so that some samples read earlier and some are a little longer.

CONCLUSION

Based on the results of the research that has been described, it can be concluded that there is a significant difference in the analysis of ABO Blood Type Agglutination Results with diluted and undiluted antisera reagents in the hospital. Gorontalo Otanaha. Shown in the experimental group (50%, 40%, and 20%) and the control group (without dilution).

a. The result of the Asymp value. Sig obtained 0.00 <0.05. Then there is a difference or Ho is rejected and Ha is accepted, meaning that there is a difference between each group (control) and the experimental group (50%, 40%, 20% dilution).
b. The results of the analysis of the kruskall wallis test obtained Chi

Square count 151.674 and Chi Square table 77.9305. This means that there is a significant effect of the treatment (control) and the experimental group (50%, 40%, and 20% dilution.

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