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COMPARISON RESULTS OF ABO BLOOD CLASS EXAMINATION AGLUTINATION WITH ANTISERA REAGENTS BEFORE EXPIRED AND ANTISERA REAGENTS EXPIRED (KADARLUARSA) IN THE LABORATORY OF REGIONAL HEALTH, GORONTALO PROVINCE

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ABSTRACT

Examination of ABO blood groups in general uses the slide method, in the examination of blood groups using antisera A and antisera reagents. Antisera reagents for blood group tests that have passed the expiration date can give less accurate results for an examination, while antisera reagents before expiration date also can provide agglutination results in accordance with a person's blood type. This type of research is quantitative analytic research with a cross sectional research design.

The sampling technique was accidental sampling with a sample size of 19 respondents from the Unexpired antisera reagent and expired antisera reagent, then compared using the Mann Whitney test.

The results showed that there was no significant difference (0.000 <0.05) between unexpired and expired antisera reagents, so it was continued in the calculation of the U value to determine the decisions of H0 and H1, the value of U1 and the value of U2 were taken the smallest values to be compared with U. table. Obtained U value table is 123. Because U count(48) <(123) then the H0 hypothesis is rejected and the H1 hypothesis is accepted with the conclusion that the results of the agglutination levels of blood group examination using unexpired antisera reagents are different from using expired antisera reagents.

Keywords:agglutination, unexpired-expired reagent, blood group

PRELIMINARY

Blood is a body fluid that is red in color and found in the closed circulatory system and is very important for human survival. Knowing a person's blood type is very important to know for medical purposes, one of which is for transfusion [2]. Blood type is important to know, which is one for the benefit of blood transfusions. Examination of ABO blood group generally uses the slide method. This method is based on the principle of reaction between agglutinogens (antigens) and agglutinins (antibodies) which form agglutinations or clots [30]. In the pre-transfusion test, the minimum that must be done in the laboratory is checking the ABO and rhesus system blood groups and cross-matching. The incidence of the ABO phenotype in each population is different,

There were 257 people who checked their blood types in the regional health

laboratory of Gorontalo province from January to December. Blood group examination is performed because it is very important to support disease diagnosis [26].

In the examination of blood groups using antisera A reagent and antisera B. Antisera reagent is a reagent used to determine a person's blood type by looking at the agglutination formed. For example, in blood group A, when the antisera reagent A and antisera B reagent were added, there was agglutination in the blood which was dripped with the antisera reagent A, while the antisera reagent B did not form agglutination [18].

Blood group antisera reagent is a reagent used to check blood type which will be reacted with a blood sample to see the results of agglutination which can determine a person's blood type. The antisera reagent used must comply with the standards such as reagents that have not passed the expiration date, reagent storage according to the procedure stated on the package, reagents that have been opened can last until the expiration date if stored at a temperature of 2-80C, if it is approaching the expiration period of one month then immediately reported to the head of the health analyst for further action, in order to provide accurate results, while a small proportion of laboratories still use blood group reagents that do not comply with reagent standards such as reagents that have passed the expired date [30].

Antisera reagent for blood group testing that has passed the expiration date can give less accurate results for a test, because it can be influenced by the factor of reagent that has expired. While the antisera reagent before expiration can also provide agglutination results that are in accordance with a person's blood type, but now many places such as health centers, clinics, and other laboratories still use expired reagents that should not be used, this use is due to economic factors. [30].

The large number of uses of blood group antisera reagents before expiration or those that have been expired in various places, the presentation obtained from the use of antisera reagents before expiration is 66.7% while the use of antisera reagents that have expired is 33.3% with different agglutination results [7]].

ABO blood group examination generally uses the slide method, which is performed to determine the type of blood group in humans. The slide method is a simple, fast and easy method for checking blood groups, because this slide method can be used in emergencies such as when examining a person's blood type for blood transfusions [30].

The ABO blood group system in humans is determined based on the type of antigen and antibody present in the blood, namely blood group A has antigen A on the surface of its erythrocytes and has antibody B in its blood serum. Blood group B has antigen B on the surface of the erythrocytes and has antibody A in the blood serum. Blood group AB has antigens A and B on the surface of the erythrocytes and does not produce antibodies A or B in the serum. Meanwhile, blood group O is without antigens, but the serum contains antibodies to antigens A and B [13].

Based on previous research the results of the presentation of the use of antisera reagents before expiration and the antisera reagent that has been expired shows different agglutination results, therefore this study was conducted to prove whether there were differences in the ABO blood group agglutination results obtained by using the antisera reagent before expired and the antisera reagent that had been expired, to determine the two reagents. more effective and get valid results to

determine the results of a person's blood group examination.

This study aims to determine the comparison of the results of ABO blood group examination agglutination with antisera reagents before expiration and antisera reagents that have been expired (expired) in the Regional Health Laboratory of Gorontalo Province. Based on the above background, researchers are interested in conducting research, entitled "Comparison of ABO Blood Type Examination Agglutination Results with Antisera Reagents Before Expired and Expired Antisera Reagents (Kadarluarsa) in the Regional Health Laboratory of Gorontalo Province ".

RESEARCH METHODS

This type of research used in this research is analytical research with a quantitative approach. Quantitative research can be defined as a research method that places more emphasis on information expressed in numbers, where these numbers represent a variable [22].

The research design used is *cross sectional. Cross sectional*is a study that connects the cause or risk and effect variables or cases that occur in the object of research and is measured or collected simultaneously (at the same time) [5].

The place for sampling and examination of this research sample was carried out at the Regional Health Laboratory of Gorontalo Province. The population in this study were all patients who had complete blood counts (Hb, HCT, PLT, RBC, WBC, ADT, RT) using EDTA tubes. The sample size was 19 samples for ABO blood group examination.

The results of the examination obtained are primary data, which is then made in tabular form using techniques univariate data analysis by describing or describing variables [4]. Then proceed with bivariate data analysis techniques to test comparative hypotheses if there is (difference or comparison) in the analysis of the results of agglutination examination of the ABO system blood group with antisera reagents before expiration and those that have expired [14]. The data analysis test used was the Mann-Whitney test.

The Mann-Whitney test is part of non-parametric statistics which aims to assist researchers in differentiating the performance results of the groups contained in the sample into two groups with two different criteria, where the level of significance used in this study is 5% (0.05) [20].

This study uses non-probability methods with sampling techniques, namely *acidental sampling,* with the technique of determining the sample based on chance, that is anyone who happens to meet the researcher can be used as a sample if the person who happens to be met is suitable as a source of data [23].

The use of the accidental sampling technique in this study is because researchers only saw differences in the results of agglutination using antisera reagents before expiration and expired antisera reagents.

The instruments used in this study were test tubes, tube racks, dropper, slide, coolbox, binocular microscope, shaker rotator. The materials used are EDTA blood, 70% alcohol, antisera reagents (Anti-A, Anti-B, Anti-D).

The procedure in the first pre-analytic stage prepares the tools and materials used, after that proceed to the analytical stage, namely the examination of the ABO blood group with the slide method by dropping 1 drop of antisera A, antisera B, and antisera D on a slide. The blood is dripped on each antisera. A, B and AB. Then homogenized using a rotator shaker, after finishing, make sure the agglutination power is macroscopic and microscopic.

The post-analytic stage is the last stage of the stage carried out to enforce the results of the tests performed, read the results of the ABO blood group agglutination examination and record the results of the agglutination.

RESEARCH RESULT

Based on the results of research conducted on September 2 to September 8 2020 at the Regional Health Laboratory of Gorontalo Province UPTD with the research title "Comparison of ABO Blood Type Agglutination Examination Results with Antisera Reagents Before Expired and Expired Antisera Reagents (Kadarluarsa) in Regional Health Laboratories Gorontalo Province ". The population size is unknown, while the sample size obtained is 19 samples for blood type (A, B, AB, and O).

Table 1. The results of the ABO blood group agglutination examination used reagents before expiration and expired reagents

No	Sampel Eksperimen	Hasil Pemeriksaan Aglutinasi	Sampel Eksperimen	Hasil Pemeriksaan Aglutinasi
		Reagen Unexpired		*Reagen expired*
1	Goldar. O	+4	Goldar. O	+2
2	Goldar. O	+4	Goldar. O	+1
3	Goldar. O	+4	Goldar. O	+1
4	Goldar. O	+3	Goldar. O	+2
5	Goldar. O	+4	Goldar. O	+1
6	Goldar. A	+4	Goldar. A	0
7	Goldar. A	+4	Goldar. A	+1
8	Goldar. A	+3	Goldar. A	+1
9	Goldar. A	+4	Goldar. A	0
10	Goldar. A	+4	Goldar. A	+2
11	Goldar. B	+4	Goldar. B	+2
12	Goldar. B	+3	Goldar. B	+2
13	Goldar. B	+4	Goldar. B	0
14	Goldar. B	+4	Goldar. B	+1
15	Goldar. B	+3	Goldar. B	+2
16	Goldar. AB	+3	Goldar. AB	+1
17	Goldar. AB	+4	Goldar. AB	+1
18	Goldar. AB	+4	Goldar. AB	+2
19	Goldar. AB	+4	Goldar. AB	+1

The Shapiro Wilk test data is said to be normally distributed if the significant value is greater than the significance level (5% or 0.05) [20].

Based on table 4.5, the normality test results for the agglutination level of ABO blood group examination on the unexpired reagent were 0.000 <0.05 and the reagent expired was 0.001 <0.05. So, it can be seen that the data obtained are not normally distributed.The results of the normality test on the agglutination level of ABO blood group examination using Unexpired and Expired antisera reagents are described in table 4.5 below:

Blood Type Agglutination	Significant	Significance Level	Ket
Unexpired	0,000	0.05	Abnormal
Expired	0.001	0.05	Abnormal

Table 2. Normality test

One of the requirements for continuing the parametric test is the Independent Sample t-Test, the data obtained must be normally distributed, while the data obtained from the normality test are all not normally distributed, so that it is continued on non-parametric analysis testing with an alternative test, namely the Mann Whitney test.

In the Mann-Whitney test (U test) the data is said to have a significant difference if the significant value (2-Tailed) is smaller than the significance level (5% or 0.05) [23]. Results of ABO blood group examination agglutination using unexpired antisera reagent and expired antisera reagent. The unexpired antisera reagent was 0.000 <0.05. Thus, it can be seen that there is a significant difference in the agglutination level of ABO blood group on the unexpired reagent. Meanwhile, the expired antisera reagent was 0.000 <0.05. So, it can be seen that there is a significant difference in the agglutination level of ABO blood group on the expired reagent. The results of non-parametric analysis on the agglutination level of ABO blood group examination using the following unexpired and expired antisera reagents:

Table 3. Mann-Whitney test

Blood Type Agglutination	Significant (2-Tailed)	Significance Level	Ket
Unexpired	0,000	0.05	Significant
Expired	0,000	0.05	Significant

From the value of U1 and value of U2, the smallest value is taken to be compared with the U table. Obtained U value table is 123. Because the value of U count (48) <(123), the hypothesis H0 is rejected and the hypothesis H1 is accepted with the conclusion that the results of agglutination levels of blood group examination using unexpired antisera reagent are different from using expired antisera reagents.

DISCUSSION

Blood group examination is an examination performed to determine a person's blood type. A person's blood classification is based on the type of antigen present on the surface of the red blood cells. Respondents who carry out blood type checks with the aim of knowing the type of blood group they have. Respondents used in this study amounted to 19 respondents according to the specified sample size. Samples from these respondents were subjected to 2 different treatments on the same sample, each sample was tested for blood groups to determine the type of blood group and saw the form of agglutination obtained from each blood group using antisera reagents before expiration and expired antisera reagents for each. -Each sample.

Reagents or often referred to as reagents are substances that play a role in a chemical reaction or are applied for analytical purposes. Reagents have uses and mostly involve chemical reactions with the help of reagents. Reagents can react chemical substances, if the reagent has passed the expiration date set by the company, the reagent content will change and can affect the analysis process of immunohematological reactions [19].

From the results of the research conducted, it was found that the results of ABO blood group examination using unexpired antisera reagents and expired antisera reagents gave different agglutination results, for unexpired antisera reagents it produced agglutination that looked like large clots with clear fluid around it with the highest degree of agglutination, namely +4 and the lowest. +3. Meanwhile, the expired antisera reagent produced agglutination which appeared to be a rather small lump with a red liquid around it with the highest degree of agglutination, which was +2 and the lowest was 0 / -.

There was a change in the degree of positive agglutination in the results of blood group tests, especially on the Anti-A examination with degrees of +3 and +4, on the Anti-B examination with agglutination degrees of +3 and +4 using unexpired blood group antisera reagents. So it can be seen that the use of reagents according to the standard of blood type testing is very important and can affect the level of agglutrination [7].

Antibodies contained in reagents will bind to antigens on red blood cells and form complex reactions. Antibodies in unexpired reagents contain clones of antibodies to other proteins. A homogeneous antibody with a specific reaction can be obtained if the antigen that enters and binds to the existing antibody. Whereas in the expired reagent the clone content contained in antibodies is getting weaker so that the specific reaction between antigen and antibody will decrease [24].

The antisera reagent that has passed the expiration date will be able to affect the agglutination result of the antigen and antibody adhesion reaction compared to the antisera reagent before the expiration date which gives a more accurate result.

Therefore, it can be seen that with the use of a suitable antisera reagent for blood group testing, the agglutination resulting from the antigen and antibody attachment reaction will not decrease and give accurate results [30].From the research results obtained from unexpired antisera reagents and expired antisera reagents, the agglutination levels varied for each respondent. This is because each respondent has a different type of blood group with different antigens and antibodies. So that when reacted with unexpired and expired reagents it gives different agglutination results.

Inappropriate use of reagents will have another impact on the results of agglutination of blood groups, namely affecting the level of agglutination resulting from antigen and antibody reactions.

In the Expired reagent, the agglutination reaction is reduced because the reaction between antibodies and multivalent (particulate) antigens will not produce cross-linking on the variation of antigen particles by antibodies. The equivalence zone is an area where there is a balance between antigen-antibody so that agglutination occurs. But the reagent expired that if the multivalent antigen, the unideterminant binds to a specific antibody, there will be a bond but no cross-linking [18].

Antisera reagent for blood group testing that has passed the expiration date can give less accurate results for a test, because it can be influenced by the factor of reagent that has expired. Many places such as health centers, clinics, and other laboratories still use expired reagents that should not be used [30].

From the research results, it was obtained that the results of agglutination using an unexpired antisera reagent were to provide complex antigen-antibody reactions resulting in good agglutination with a +4 degree of agglutination, while expired antisera reagents gave weakened antigen-antibody reactions resulting in specific reactions, between antigen and antibody. will be reduced, resulting in poor agglutination with a degree of agglutination +2 to 0 / -.

Agglutination in blood group examination is the adhesion of red blood cells caused by antibodies attached to the antigens of several red blood cells, causing a webbing that can entangle the cells into clusters. Agglutination itself has a degree of agglutination degree ranging from +1 to +4, which will determine the results of agglutination examination of ABO blood group [16].

The results of agglutination levels in the Mann Whitney test were significant (0.000 <0.05) in the Unexpired and Expired antisera reagent groups, after that it was continued to compare the calculated U value obtained with the U table to test the null hypothesis (H0) and hypothesis one (H1). From the value of U1 and value of U2, the smallest value is taken to be compared with the U table. Obtained U value table is 123. Because the value of U count (48) <(123) Based on these results, the hypothesis can be answered, namely the hypothesis H0 is rejected and the hypothesis H1 is accepted with the conclusion that the results of the agglutination level of blood group examination using unexpired antisera reagent are different from using reagents. antisera expired.

Blood group antisera reagent is a reagent used for blood group examination which will be reacted with a blood sample to see the results of agglutination. The antisera reagent used must comply with standards such as reagents that have not passed the expiration date, reagent storage according to the procedure stated on the package, reagents that have been opened can last until the expiration date if stored at a temperature of 2-80C [30].

CONCLUSION

Based on the results of the research previously described, it can be concluded that there is a significant difference between the agglutination levels of ABO blood group using antisera reagents before expiration and expired antisera reagents caused by the use of reagents that trigger changes in the degree of agglutination and are shown in the results of the mann test values. whitney (0.000 <0.05) between unexpired and expired results. So it is stated that there is a significant difference between the agglutination levels of ABO blood group using antisera reagent before expiration and expired antisera reagent. The result of the U count is compared with U table. U value count (48) <

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