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The Relationship Between Phlebotomists' Knowledge, Attitudes, and Behaviors Regarding Venous Blood Specimen Collection and Handling with Laboratory Specimen Quality at RSUD X

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A B S T R A C T

Background: Errors in blood specimen collection and handling can lead inaccurate, misinterpretation of laboratory test results. However, the urgent crossmatching, sometimes even omitting the incubation phase. This accelerated process may compromise the accuracy of results.

Purpose: To determine the relationship between phlebotomists' knowledge, attitudes, and behaviors regarding blood specimen collection and handling with the quality of venous blood specimens and evaluate the impact of incubation on crossmatch results at RSUDX.

Methods: Data analysis used the Chi-square test. A quasi-experimental design with a one-group pre-test and post-test design was adopted for this study and the Wilcoxon signed-rank test was conducted to assess for statistically significant differences.

Resut: There was a relationship between phlebotomists' knowledge of blood specimen collection and handling and the quality of laboratory specimens (p-value 0.001). There was no relationship between phlebotomists' attitudes toward blood specimen collection and handling and the quality of laboratory specimens (p-value 0.682). There was no relationship between phlebotomists' behavior in blood specimen collection and handling and the quality of laboratory specimens (p-value 0.494). Both incubated and non-incubated crossmatch tests shows same result. Wilcoxon signed-rank test showed p-value<0.05 for the incubated group and an asymptotic significance (2-tailed) of 1.000(P>0.05). Similarly, the non-incubated group p-value is 0.000 (p<0.05) and an asymptotic significance (2-tailed) of 1.000 (p>0.05).

Conclusion: is a significant relationship between phlebotomists' knowledge and the quality of laboratory specimens. There is no relationship between phlebotomists' attitudes and behaviors regarding blood specimen collection and the quality of laboratory specimens at RSUD X. There was no significant difference results between incubated and non-incubated sample

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INTRODUCTION

A clinical laboratory is a laboratory that carries out clinical specimen examination services in the fields of hematology, clinical chemistry, microbiology clinical, clinical parasitology, and clinical immunology [1]. Specimens that are often used as laboratory examination materials are blood specimens. Blood (*whole blood*) is one of the biological specimens taken from humans through a blood drawing technique known as phlebotomy which is carried out by a phlebotomist [2]. The venous blood drawing technique is a technique that is often used because the use of venous blood specimens is often requested for laboratory examinations with the aim of establishing a diagnosis, monitoring treatment and for therapy of certain diseases [3].

Specimens to be examined in the laboratory must meet the requirements according to the type of examination. Specimen rejection is carried out when it is found that the specimen is unsuitable for examination, including a mismatch between the identity of the examination form and the specimen, the tube has no identity, hemolysis, the wrong time of specimen collection, the specimen is contaminated with infusion fluid, insufficient blood volume, use of the wrong specimen tube, agglutination is found in examinations that require blood or plasma [4].

Improper blood specimen collection and handling procedures can result in inaccurate examinations that result in misinterpretation of laboratory test results. These errors are known as pre-analytical errors and account for approximately 70% of all errors in laboratory testing [5].

A study conducted by Sushma at the clinical biochemistry laboratory, CIMS, Bilaspur showed that out of 19,411 specimens, 670 specimens or 3.45% were rejected for examination due to errors in the pre-analytical stage [6]. Another study conducted by Najat (2017) at the Iraqi Clinical Laboratory explained the prevalence of improper specimen handling during the pre-analytical stage of 39% with the main reasons for errors occurring in hemolyzed samples (9%), misidentification of specimens (8%) and the occurrence of frozen specimens (*clotted*) by 6% [7].

According to Thimesch (2016) the quality of the specimen is greatly influenced by the method of taking, collecting and handling the specimen. Blood collection is the initial stage and plays an important role in the series of processes to the accuracy of laboratory examination results. Thus it can be said that good specimen quality is obtained from a good pre-analytical stage and this is influenced by the level of knowledge of the phlebotomy officer himself [8].

Based on research conducted by Indyanty (2015) showed that there is a significant positive correlation between nurses' knowledge of phlebotomy and specimen quality. This means that the better the nurses' knowledge of phlebotomy, the better the quality of the specimens obtained [9]. Another study conducted by Mahyaningsih (2018) in the Bandung City Hospital laboratory, showed that the knowledge factor of officers affects specimen quality. Therefore, good knowledge of specimen collection and handling must be mastered by phlebotomists [10].

In reality, in the Laboratory of RSUD X, there are still blood specimen rejections because the blood received is not suitable as a test material. The rejection of the specimen is caused by various reasons including hemolysis specimens, frozen specimens because they were not immediately homogenized, and the volume of the specimen does not meet the criteria. The quality of specimens that do not meet these requirements can cause errors in laboratory results which have an impact on misinterpretation of examination results, resulting in errors in diagnosis, follow-up and treatment decisions [11].

Data on the level of knowledge, attitudes and behavior of phlebotomists about the collection and handling of venous blood specimens at RSUD X are currently not available and have never been evaluated. Therefore, researchers need to conduct a study entitled "The Relationship between Knowledge, Attitudes

and Behavior of Phlebotomists about the Collection and Handling of Venous Blood Specimens with the Quality of Laboratory Specimens at RSUD X".

Thalassemia is a disorder of hemoglobin synthesis caused by mutations in or near the globin gene that are inherited *and* are able to produce chains of certain globin or have a decreased rate of synthesis. Decreased production of hemoglobin (Hb) synthesis will affect the quality of life of red blood cells and accelerate the death of red blood cells [12]. Since 2012, there have been 4,896 cases until June 2021, data on people with Thalassemia in Indonesia were 10,973 cases [13].

In its handling, the management of thalassemia until now is still in the form of lifelong blood transfusions. Repeated blood transfusion management will cause transfusion reactions such as shivering, urticaria, anaphylactic shock, and can even cause organ dysfunction due to accumulation of excess iron [12]. In more severe Thalassemia, anemia occurs and in the most severe Thalassemia it can be fatal or lifelong dependent on repeated blood transfusions [13].

One of the preparations that must be done before transfusion is compatibility testing. Compatibility testing aims to prevent hemolytic transfusion reactions consisting of blood type examination (ABO and Rhesus), antibody screening, and cross-matching. *Crossmatching examination* aims to determine whether the donor's erythrocyte antigens match the antibodies in the patient's serum and the patient's erythrocyte antigens to the antibodies in the donor's serum [14].

The Cross-matching gel test method is a method for detecting the reaction of red blood cells with antibodies. Referring to the results of research conducted by McCullough (2012) which states that the gel test method provides more convenience and accuracy than the tube test method. The gel test method has many advantages over the tube test method. In addition to saving examination time, the test procedure is also simpler and reading the results is easier [15]. The washing process and addition of CCC (Coomb's Control Cell) are no longer carried out. In crossmatch examination, there are factors that can affect the occurrence of antigen and antibody reactions, such as temperature and incubation time. The use of optimal temperature can shorten the incubation time. Temperature is also an indication of clinical significance as a determinant of the presence of irregular antibodies, such as the presence of alloantibodies that make it difficult to obtain suitable donor blood. Clinically significant antibodies mainly refer to irregular alloantibodies that react at a temperature of 37 °C [16]. If the incubation time is too short, it will cause the Ag and Ab reactions to weaken, while too long an incubation time will cause dissociation of antibodies.

Some problems that often occur in BDRS are crossmatch without incubation. This procedure is carried out because of the need for urgent transfusion of thalassemia patients so that pre-transfusion testing is accelerated. If this crossmatch procedure is carried out without incubation in thalassemia patients, the risk is that hemolysis will occur, namely the antigen that enters the patient's body is recognized as a foreign antigen by the body, causing the transfused blood to lyse.

METHOD

This study is an analytical observational study using a questionnaire instrument. The sample of the study was 126 nurses and 14 ATLM who performed phlebotomy at RSUD X. The data analysis used was the *Chi-square test*.The type of research used in this study is *experimental research*. *Experimental research* is an experimental activity that aims to determine a symptom or effect that arises as a result of a certain treatment [17]. The experiment used is a *quasi experiment. Quasi experiments* provide *a pretest* or initial observation before being given an intervention, after which an intervention is given and then *a posttest* or final observation is carried out [18].

The work procedures carried out in this study include sample preparation, crossmatch examination, analysis of examination results by comparing the crossmatch results of thalassemia patients with the

interpretation of the manufacturer's standard coombs test results. Data obtained from the crossmatch examination results are presented in table form and then statistical tests are carried out.

RESULTS AND DISCUSSION

a. The Relationship between Phlebotomist Knowledge on Venous Blood Specimen Collection and Handling with Laboratory Specimen Quality at X Regional Hospital

Table 1. Cross Tabulation of the Relationship between Phlebotomist Knowledge on Taking and Handling Venous Blood Specimens and the Quality of Laboratory Specimens at RSUD X

Knowledge	Venous Blood Sample Quality						*ρ- Value*
	Worthy		Not feasible		Total		
	Σ	%	Σ	%	Σ	%	
Good	96	68.6	9	6.5	105	75.0	0.001
Enough/Not Enough	23	16.4	12	8.6	35	25.0	
Amount	**120**	**85.7**	**20**	**14.3**	**140**	**100.0**	

Source: primary data, processed in 2024

Based on table 1, it shows that most of them have good knowledge and produce specimens with decent specimen quality, which is 68.6%. The results of the *Chi-square* test on the SPSS program show that there is a relationship between phlebotomy knowledge and the quality of venous blood specimens produced, with a significance value of *ρ - value* 0.001 <0.05.

b. The Relationship of Phlebotomist Attitudes Regarding the Collection and Handling of Venous Blood Specimens to the Quality of Laboratory Specimens at X Regional Hospital

Table 2. Cross Tabulation of the Relationship between Phlebotomist Attitudes on Taking and Handling Venous Blood Specimens and the Quality of Laboratory Specimens at RSUD X

Attitude	Venous Blood Sample Quality						*ρ- Value*
	Worthy		Not feasible		Total		
	Σ	%	Σ	%	Σ	%	
Good	88	62.9	17	12.1	105	75.0	0.682
Enough/Not Enough	31	22.1	4	2.9	35	25.0	
Amount	**120**	**85.7**	**20**	**14.3**	**140**	**100.0**	

Source: primary data, processed in 2024

Based on table 2, it shows that out of 140 respondents, most of them have good category attitudes that can produce decent specimen quality, which is 62.9%. The results of the *Chi-square* test on the SPSS program show that there is no relationship between the phlebotomy attitude variable and the quality of the venous blood specimen produced. This can be seen from the significance value of *p-value* 0.682> 0.05.

c. The Relationship of Phlebotomist Behavior in Collecting and Handling Venous Blood Specimens to the Quality of Laboratory Specimens at RSUD X

Table 3. Cross Tabulation of the Relationship between Phlebotomist Behavior in Collecting and Handling Venous Blood Specimens and the Quality of Laboratory Specimens at RSUD X

Behavior	Venous Blood Sample Quality						*ρ- Value*
	Worthy		Not feasible		Total		
	Σ	%	Σ	%	Σ	%	
Good	91	65.0	14	10.0	105	75.0	0, 494
Enough/Not Enough	28	20.0	7	5.0	35	25.0	
Amount	**120**	**85.0**	**20**	**15.0**	**140**	**100.0**	

Source: primary data, processed in 2024

Based on table 3 shows that out of 140 respondents, most of them have good category behavior in getting decent specimen quality, which is 65.0%. The results of the *Chi-square test* on the SPSS program show that there is no relationship between phlebotomist behavior and the quality of venous blood specimens. This can be seen from the significance value of *ρ - value* 0.494 > 0.05.

Based on the frequency distribution of phlebotomists according to the level of knowledge about taking and handling venous blood specimens at RSUD X, the results showed that most respondents had a level of knowledge in the good category, which was 75.0%. This shows that in general phlebotomists at RSUD X have good knowledge about taking and handling venous blood specimens. Good knowledge of blood specimen collection and handling procedures is key to ensuring that the specimens taken are correct and processed correctly. The laboratory must obtain good quality specimens so that the results obtained describe the patient's condition as it should be and produce good quality examinations [19]. Therefore, good knowledge of specimen collection and handling must be mastered by a phlebotomist.

Knowledge is something related to the learning process. Various internal factors, such as motivation and external factors in the form of available information suggestions, as well as socio-cultural conditions can influence the learning process. A person can gain knowledge naturally or through intervention, either directly or indirectly, one of which is through training [20]. According to Notoatmodjo (2014), training is needed to improve a person's abilities. Through training, a person can gain new information and knowledge that is relevant to their field or task, helping to update and improve existing competencies, especially in dealing with changes in technology or new work methods [17]. This is in line with research conducted by Sushma and Srikant (2019) showing that there was a decrease in the frequency of errors in the pre-analytical stage before and after training was carried out on staff [6].

The majority of respondents, including nurses and ATLM, agreed on the importance of completing laboratory request forms with essential patient information (96.4%) and ensuring proper labeling on tubes with at least the patient's name, date of birth, and MR number (92.1%). Most respondents acknowledged that specimen collection and handling methods influence specimen quality (85.0%) and that shaking during homogenization can cause hemolysis (77.1%). Additionally, 80.7% agreed that phlebotomists must understand the types and functions of anticoagulants based on tube color. However, only 57.9% agreed that excessive pressure on the syringe plunger when transferring blood into tubes causes hemolysis, suggesting a lack of awareness among respondents, potentially due to habitual practices during venous blood collection.

Most respondents disagreed with several incorrect practices, such as performing venous punctures while the alcohol is still wet (82.1%), leaving a tourniquet in place for more than one minute (77.1%), addressing clots in blood specimens by manually removing them from the tube (82.9%), and skipping the homogenization process for hematology and coagulation specimens under the assumption it does not affect results (52.9%). The findings align with field observations, particularly for EDTA blood specimens collected by nurses, which often show improper mixing of anticoagulants due to a lack of homogenization. This suggests that such practices may have become habitual among respondents. One of the factors that influences attitudes according to Robbins in Fauzan (2018) is habit [21]. What is often done in everyday life, whether it is positive or negative, will become a person's attitude in carrying out everyday life. According to Wawan and Dewi (2011), attitudes are not innate but are formed or learned throughout development in relation to their objects. Attitudes can change therefore attitudes can be learned and attitudes can change in people if there are circumstances [22].

Based on the behavior of phlebotomists regarding the collection and handling of venous blood specimens at RSUD X, the results showed that most respondents had behavior in the good category, which was 75.0%. This shows that in general phlebotomists at RSUD X already have good behavior regarding the procedures for taking and handling venous blood specimens. It can be concluded that most phlebotomists at RSUD have complied with the Standard Operating Procedure for taking and handling venous blood specimens.

Behavior is formed from various experiences and interactions between humans and their environment [23]. Behavior is a factor within oneself that can influence a person's compliance. A person who has positive behavior can comply with a rule, in this case, compliance in carrying out standard operating procedures for taking and handling blood specimens. Green's theory quoted by Damayanti (2017) states that factors that can influence behavior include predisposing factors including knowledge, attitudes, beliefs, beliefs, values and so on [24]. Supporting factors include the physical environment, the availability or unavailability of work safety facilities or facilities, for example the availability of supporting tools, training and so on. Reinforcing factors include laws, regulations, supervision

The quality of laboratory specimens refers to the extent to which biological samples or examples taken from patients meet the criteria required for accurate and reliable laboratory analysis. Specimens to be examined in the laboratory must meet the requirements in accordance with the type of examination, the accuracy and adequacy of the specimen volume, the nature of the specimen that is worthy, namely not hemolyzed, the use of appropriate anticoagulants, stored in the right container and must be identified and verified the patient's identity [8]. According to Manik and Haposan (2021) phlebotomy errors in handling blood samples will result in errors in the analysis of test results, which can lead to misinterpretation of results and inappropriate patient management. This Errors also lead to additional laboratory tests or other tests that are not needed by the patient [25].

Pre-analytics is an initial preparation stage that greatly determines the quality of the specimens to be produced and affects the next process. Valid and accurate results are largely determined by how the blood specimen is taken and handled by the phlebotomist. Errors or inaccuracies in the pre-analytic process contribute to approximately 70% of all errors in laboratory diagnosis [5]. Previous research conducted by Sushma (2019) at the clinical biochemistry laboratory, CIMS, Bilaspur showed that out of 19,411 specimens, 670 specimens or 3.45% were rejected for examination due to errors in the pre-analytic stage [6]. This study found that the quality of the specimens was not suitable, namely 1.30%, including hemolysis specimens (0.44%), insufficient specimen volume

(0.37%), specimen identity mismatch (0.34%), frozen specimens (0.12%), and tube errors (0.03%). According to Sari I (2023), some of the discrepancies could have occurred due to the ratio between blood volume and anticoagulant. Blood volume less than the amount of anticoagulant can cause blood specimens to clot [26]. In this study, it was found that the majority of the quality of venous blood specimens produced by phlebotomists at RSUD X produced specimen quality in the appropriate category (98.7%).

Based on table 5, it shows that most respondents who have good knowledge get a decent sample quality of 68.6%. The results of the *Chi- square test* show that there is a relationship between respondents' knowledge regarding the collection and handling of venous blood specimens and the quality of specimens produced by respondents in the laboratory of RSUD X with *a p-value of* 0.001. This is in line with research conducted by Indyanty et al (2015) which states that there is a significant positive correlation between nurses' knowledge about phlebotomy and specimen quality [9]. Anwar's opinion quoted by Faridah (2020) states that the knowledge possessed by health workers is an important factor in forming behavior [27]. This means that the knowledge possessed by a phlebotomist can influence actions in taking blood.

This study found no significant relationship between phlebotomist attitudes toward venous blood collection and handling and the quality of specimens produced, as indicated by a Chi-square test (p-value 0.594 > 0.005). The average attitude scores for producing acceptable and unacceptable specimen quality were similar, reflecting that phlebotomists generally have good knowledge and practices, resulting in acceptable specimen quality. This study is in line with the study conducted by Indyanty et al (2015) which states that there is no correlation between nurses' attitudes about phlebotomy and the quality of the specimens produced [9].

This study also found no relationship between phlebotomist behavior regarding the collection and handling of venous blood specimens with the quality of specimens produced by respondents in the laboratory of RSUD X. This can be seen from the significance value of the *Chi-square* test results in the SPSS program showing ρ - *value* 0.412 > 0.05. This study is in line with research conducted by Indyanty et al (2015) which states that there is no correlation between nurse behavior during phlebotomy and specimen quality [9]. As professionals, ATLMs should exhibit good attitudes and behavior by prioritizing quality in laboratory examinations and sharing their knowledge and experience effectively [28].

Errors in the venous blood specimen collection stage occur due to those related to specimen quality, non-compliance with phlebotomy SOPs, heavy workloads, lack of training or supervisor attention to ensure the quality of pre-analytical stage samples before laboratory examination [26]. Phlebotomy training for ATLM and standardization of phlebotomy practices can improve specimen quality [29].

This study faced limitations due to the inability to directly supervise respondents while completing the questionnaire, given their large number and distribution. Although the researcher provided explanations and was available for questions, some responses may not accurately reflect the respondents' conditions.

This study was conducted at the Blood Transfusion Unit of Jampangkulon Hospital with the subjects of the study being thalassemia patients who underwent blood transfusions during January 2024 at the Thalassemia Polyclinic of Jampangkulon Hospital. The number of samples in this study was 25 thalassemia patients with different treatments in the *crossmatch examination.* namely incubated in *an id-incubator* at a temperature of 37 ° C for 15 minutes and without incubation.

The results of the description of the characteristics of thalassemia patients in this study based on gender showed that most patients were female, namely 60% or 15 patients. While a small portion of patients were male, namely 40% or 10 patients. Meanwhile, the results of the description of the characteristics of thalassemia patients based on age showed that most thalassemia patients were in the age range of 6-10 years, namely 52% or 13 patients. While a small portion of patients were in the age range of 0-5 years, namely 20% or 5 patients.

This study involved specimens from thalassemia patients who had undergone repeated blood transfusions with various blood types (A, B, O, AB) and rhesus positive. Each 1 mL specimen was tested for blood type, rhesus, and crossmatch compatibility under two conditions: without incubation and after incubation at 37°C for 15 minutes. The results of the crossmatch examinations at Jampangkulon Hospital are described below. The results of the *crossmatch examination of the gel test* method on 25 samples of thalassemia patients obtained varying interpretations of the results with the same agglutination *grade* in each sample that was incubated at 37°C for 15 minutes or without incubation. Samples with information 0 mean *compatible*, namely there is no agglutination in *the microtube*. While samples with information 1 and 2 mean *incompatible*, namely there is agglutination in *the microtube* with an agglutination grade of 1+ and 2+. Whereas according to research by Arrosyada, et.al (2023), the average difference in blood results that are delayed and not delayed by cross-examination with the gel method is 45% of changes in results [30].

The Normality Test is a test conducted with the aim of assessing the distribution of data in a group of data or variables, whether the data distribution is normally distributed or not. The normality test of this research data uses the *Shapiro-Wilk test.* The complete results of the normality test can be seen in table 2 below:

Table 2. *Crossmatch* Examination Normality Test

	Shapiro-Wilk	***p-value***	**Information**
Without Incubation	0.731	0.000	Not normally distributed
Incubation	0.731	0.000	Not normally distributed

Based on table 2, the data shows that the *crossmatch examination* without incubation obtained *a p-value* of 0.000 (p <0.05) and the *crossmatch examination* with incubation for 15 minutes obtained *a p-value* of 0.000 (p <0.05). This means that the data from this study are not normally distributed. Therefore , Further analysis tests were carried out using non-parametric statistical tests, namely the *Wilcoxon test.*

According to Sugiyono (2017), *the Wilcoxon Signed Rank Test* or also called *the Wilcoxon Match Pair* is a non-parametric test to analyze the significance of differences between two paired ordinal-scale data but distributed abnormally [31]. The results of the normality test in this study were that the data was not normally distributed, therefore the test was continued with the *Wilcoxon test* . The following is table 3 containing the SPSS *output* for *crossmatch* examination *ranks* with and without incubation .

Table 3. *Crossmatch* Check Ranks

	N	***Mean Ranks***	***Sum of Ranks***
Negative Ranks	0	0.00	0.00
Positive Ranks	0	0.00	0.00
Ties	25		
Total	25		

Based on table 3 above, it can be concluded that the *negative ranks* or negative differences and the *positive ranks* or positive differences between the *crossmatch examination* without incubation and with incubation, both in the N value, *Mean Ranks* and *Sum of Ranks,* indicate that there is no difference in the examination results. Meanwhile, *Ties* is the similarity between the *crossmatch examination* without incubation and with incubation. The Ties value is 25, which means that there is a similarity between the two treatments for the examination sample. After *the output* examination *ranks* , the next is the *Wilcoxon* test *output for crossmatch* examination with incubation and without incubation, more details are in table 4 below.

Table 4. *Wilcoxon Crossmatch* Test

	Incubation - Without Incubation
Z	0.000
Asymp. Sig. (2-tailed)	1,000

Based on table 4 above, it shows that *Asymp. Sig. (2-tailed)* is worth 1,000 greater than 0.05, so it can be concluded that the hypothesis is rejected, which means that there is no significant difference in the *crossmatch examination data*. Based on the results of the statistical calculations above, it can be concluded that there is no difference in the *crossmatch results* of the gel test method with incubation and without incubation in thalassemia patients at Jampangkulon Hospital.

Based on the research conducted, there is a picture of the results of the crossmatch examination without incubation, namely compatible results in 11 specimens which are indicated by the absence of agglutination occurring in the major, minor and autocontrol where all red blood cells are at the bottom of the microtube. While in the other 14 specimens showed incompatible results which means agglutination occurred in the minor and autocontrol where some red blood cells were at the bottom of the microtube and some others went to the middle to the top of the microtube.

In the crossmatch examination with incubation at 37°C for 15 minutes, compatible results were obtained in 11 specimens which were indicated by the absence of agglutination occurring in the major, minor and autocontrol where all red blood cells were at the bottom of the microtube. While in the other 14 specimens showed incompatible results which meant agglutination occurred in the minor and autocontrol where some red blood cells were at the bottom of the microtube and some others went to the middle to the top of the microtube. The following is a description of the results of the crossmatch examination carried out.

Figure 1 Crossmatch examination results

Based on the image above, there are the same results in the incubation treatment and without incubation of the crossmatch examination sample. In reading the interpretation of the results, it

shows a picture of agglutination at the same reading grade. However, the crossmatch examination that was given incubation treatment showed slightly more agglutination compared to the results of the crossmatch examination without incubation. Observations made after a certain incubation time are expected to cause the reaction to take place perfectly and produce the expected agglutination. Crossmatch examination without incubation at a temperature of 37 °C allows the formation of a maximum antigen antibody reaction. When carrying out the incubation stage for an examination, the optimum incubation time must be appropriate and precise [32].

Agglutination observed more frequently after incubation likely occurs because antibodies and antigens react effectively only after 15 minutes at 37°C using the ID-incubator gel test. If antibodies and antigens are incompatible, no agglutination occurs, even after centrifugation [32].

The results of this study are in line with the results of research conducted by Fadillah et al. (2023), namely that there was no effect of temperature variations and incubation time on the *crossmatch examination of the gel test* method [16]. However, in contrast to the compatibility results that occurred, in the study of Fadillah et al. (2023) all samples studied obtained *compatible results* which showed negative agglutination results in major, minor and autocontrol examinations where red blood cells were at the bottom of *the microtube* [16]. This happens because the samples used are likely to come from normal people and there are no irregular antibodies in the patient and donor blood specimens. Another possibility that can occur is that researchers minimize the influence that will occur on the research results if carried out on normal patients without blood disorders. So that varying the temperature and incubation time in the *crossmatch examination of the gel test* method in the study of Fadillah et al. (2023) does not affect the results [16]. In Fermadani's study (2017), positive results were found in samples that were not incubated, while in the incubation treatment, samples with negative agglutination results were obtained [33]. Repeated blood transfusions can result in the production of alloantibodies against one or more red blood cell (RBC) antigens, which complicates subsequent transfusions. Alloantibodies can interfere with crossmatch testing and therefore can cause delays in obtaining compatible blood and are sometimes also associated with delayed-type hemolytic transfusion reactions [34]. *Incompatible results in this crossmatch* examination do not interfere with the transfusion process in thalassemia patients because agglutination reactions occur in minor and autocontrol. According to the Minister of Health Regulation number 91 of 2015 concerning blood transfusion service standards, the interpretation of the results of the crossmatch test is compatible with incompatible blood only concentrated red blood cells are transfused with the note that the minor gradation is the same as or lower than the autocontrol gradation. In this study, the *crossmatch results* between minor and autocontrol have the same *grade so that the donor blood is still allowed to be transfused to thalassemia patients.*

Other research highlights factors influencing antigen-antibody reactions beyond the absence of irregular antibodies. These reactions occur in two stages: first, antibodies bind to red blood cell surfaces; second, they interact, causing cells to approach and agglutinate. The first stage is affected by factors such as temperature, antibody affinity, pH, incubation time, antigen-antibody ratio, and ionic strength. The second stage depends on molecule charge, cell spacing, membrane properties, and molecular structure. The freshness of serum, plasma, and red blood cells also plays a critical role, with fresh samples yielding optimal reactions. For crossmatch examinations, it is recommended to use fresh red blood cells or store serum/plasma at -20°C or lower if testing is delayed [16].

This study has limitations in the form of the number of specimens studied. With more samples and variations in blood types with an even number, it is possible to provide a picture of other results in *crossmatch examinations* with incubation and without incubation in thalassemia patients.

Therefore, in further research it is expected to be carried out on a larger number of samples and more varied blood types with an even number of samples.

CONCLUSION

Based on the research results, the following conclusions can be drawn:

1. Most phlebotomists at RSUD X have a good level of knowledge (75%), a small number have sufficient (22.1%) and insufficient (2.9%) knowledge regarding the collection and handling of venous blood specimens. Most phlebotomists at RSUD X have a good attitude (75%), a small number have attitudes in the sufficient category (24.3%) and insufficient (0.7%) regarding the collection and handling of venous blood specimens. Most phlebotomists at RSUD X have good behavior (75%), a small number have behavior in the sufficient category (23.6%) and insufficient (1.4%) regarding the collection and handling of venous blood specimens.
2. Most of the quality of venous blood specimens obtained from phlebotomists at RSUD X was categorized as suitable (98.7%) and a small portion was categorized as unsuitable (1.3%).
3. There is a relationship between phlebotomist knowledge regarding the collection and handling of venous blood specimens and the quality of laboratory specimens at RSUD X. significance *p-value* 0.001 < 0.05. There is no relationship between phlebotomist attitudes regarding the collection and handling of venous blood specimens with the quality of laboratory specimens at RSUD X with a significance *p-value of* 0.682 > 0.05. There is no relationship between phlebotomist behavior regarding the collection and handling of venous blood specimens with the quality of laboratory specimens at RSUD X with a significance *p-value of* 0.494 > 0.05.
4. *Crossmatch* examination of the *gel test* method with an incubation time of 15 minutes at 37°C and without incubation in thalassemia patients calculated using the *Wilcoxon test* shows that *Asymp. Sig. (2-tailed)* is worth 1,000 greater than 0.05, which means that the hypothesis is rejected. This means that there is no difference between *crossmatch examination* without incubation and *crossmatch examination* with incubation on 25 specimens of thalassemia patients at Jampangkulon Regional Hospital.

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