

## Analysis of The Results of The Rhesus System Blood Group Phenotyping Examination in Routine Type O Blood Donors

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### Abstract

The Rhesus (Rh) system is the second most important blood group system after ABO, has the highest immunogenicity and tends to produce alloantibodies that cause most transfusion reactions. In the Rh blood group system, there are more than 55 antigens, but the main ones are D, C, E, c, and e. Currently, only the D antigen is carried out without examining four other important Rh antigens. Still scarce, studies on the proportion of Rh antigens, except D, in Indonesia; in this study we have determined Rh antigens and phenotypes among a population of routine blood donors. The purpose of this study was to determine the phenotypic variation and frequency distribution of the main antigens of the Rhesus blood group system in routine blood type O blood donors at UDD PMI Depok City. This study was descriptive and observational with a cross-sectional design. Primary data are obtained from the Rhesus system antigen examination results on donor blood samples. The sample was 140 blood samples from 140 routine donors. A rhesus phenotyping examination was carried out using the gel method. The study was conducted on 140 routine donor blood samples at UDD PMI Depok City. The characteristics of the subjects were male (69.29%) and female (30.71%), with an average age of 38 years. Samples are analyzed for five major Rhesus antigens. Rhesus antigen test shows D antigen (100%), e antigen (97.14%), C antigen (95.71%), c antigen (36.43%) and E antigen (27.86%). Rhesus phenotype results were R<sub>1</sub>R<sub>1</sub> (DCe/Dce) (62.86%), R<sub>1</sub>R<sub>2</sub> (DCe/DcE) (22.86%), R<sub>1</sub>r (DcE/dce) (9.29%), R<sub>2</sub>R<sub>2</sub> (DcE/DcE) (2.86%), R<sub>2</sub>r (DcE/dce) (1.43%), and R<sub>1</sub>R<sub>2</sub> (DCe/DCE) (0.71%). In conclusion, the frequency of phenotypic changes in the Rh blood group system of regular blood donors at UDD PMI Depok City is classified as the most common types: DCe/DcE, DcE/DcE, DCe/dce, DcE/DcE, DcE/dce and DCe/DCE.

**Keywords** *alloantibodies, phenotyping, Rh antigen*

### INTRODUCTION

Each individual has erythrocyte antigens that are unique to their membranes. More than 345 erythrocyte antigens can divide blood into 43 group systems. The Rhesus (Rh) system is the second most important blood group system after ABO, has the highest immunogenicity and tends to produce alloantibodies that cause most transfusion reactions. This is because Rhesus antigens are highly immunogenic, especially the D antigen. The Rhesus system, which includes the D, C, c, E, and e antigens, differs from the ABO system in several ways. Antibodies to these antigens do not occur as naturally as ABO antibodies, but when stimulated, they can cause an acute hemolytic reaction that can be fatal (Choate, 2018).

Rh D antigen testing is required in pretransfusion in donor blood because of its immunogenicity. Of the 50 antigens in the Rh system, the main ones are D, C, E, c, e. Rh testing on donated blood is performed in some countries; for example, Karim et al.'s (2015) study in Pakistan showed that the most important phenotype was DCe 44%. Similar research was also conducted by Gundrajukuppam et al. (2016) and Garg et al. (2015) (Thakral et al., 2010).

The reason for the phenotypic matching between donor blood and patient blood is to prevent the formation of alloantibodies and the subsequent negative consequences of hemolytic transfusion reactions. Currently, phenotype matching is only applied to specific patient populations, such as patients requiring multitransfusion (Peck, 2019).

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The problem that occurs is that pretransfusion examination in Indonesia is currently new ABO and Rhesus D blood group antigen tests are carried out only C, E, c, and e antigen tests are not systematically performed, even though other Rh antigen tests (C, E, c and e) in pre-transfusion testing are useful for preventing alloimmunization, especially for multitransfusion patients (Dewi et al., 2019). Therefore, it is necessary to carry out additional erythrocyte examinations both patients and donors to determine the blood antigens of patients and donors so that donor blood is prepared in accordance with the patient's erythrocyte antigens so that alloantibodies do not arise. Of the 211 UTDs in Indonesia, one of them is UTD PMI DKI Jakarta, which has carried out tests on Rh (C, E, c, and e) antigens, specifically meet the patient's blood needs with alloantibodies and is still limited to group O only with the consideration that PRC O can be for anyone (Gantini et al., 2019). PMI Depok City Blood Donor Unit is a unit that serves blood demand in Depok and surrounding areas. Based on data from the UDD management information system of PMI Depok City, the number of routine blood donations from 2022 until August 2023 is 47,323. From these data, all donors (100%) were Rhesus positive with the distribution of blood group A (12,336), group B (13,170), group O (18,167) and group AB (3,650). Blood type O is reported as the most common blood type.

The purpose of this study was to determine the phenotypic variation and frequency distribution of the main antigens of the Rhesus blood group system in routine blood type O blood donors at UDD PMI Depok City. Based on the data findings from this study, it is expected to provide information about the phenotype of the Rhesus system in donor blood and can be used as a reference in giving transfusions to patients with the appropriate Rhesus system phenotype, to reduce the risk of alloantibody formation in patients who receive transfusions. Blood transfusions and alloimmune reactions can be minimized when blood transfusion safety is ensured.

## LITERATURE REVIEW

The Rhesus system is the second most important classification system in pretransfusion examination. The presence of D antigen is called Rh positive, while a person without D antigen is called Rhesus negative. E/e and C/c are two pairs of allele antigens found in the Rh protein. The genes Rh, E/e, D, and C/c are arranged sequentially on chromosome 1 and form haplotypes, such as cDE or Cde. Two haplotypes can be produced by the expression of two to five Rh antigens (Wah et al., 2020). Although the Rhesus genes and phenotype are stable throughout life, their frequency varies across races and geographical boundaries. These genes and phenotypes also have different biochemical compositions. Polymorphisms in this blood group system are important in the genetic study of populations, in evaluating the possibility of hemolytic disease in newborns, in resolving cases of paternity disputes and for forensic purposes (Chandekar et al., 2017).

Four other Rh antigens, namely C and c, E and e, are the main antigens of the Rhesus system, the most significant being two pairs of alleles, namely Cc and Ee (Fung et al., 2014). Rhesus antigens are proteins that span the membrane of erythrocytes. Antibodies to these antigens do not occur naturally but, when stimulated, can cause an acute hemolytic reaction that can be fatal (Choate, 2018). Just like the ABO system, the Rh antigen is located on the surface of erythrocytes. In contrast to the ABO system, the primary Rh antigen is found exclusively on erythrocytes and not on tissue cells or in soluble form in body fluids (Harmening, 2018).

Examination phenotyping is intended for patients who already have alloantibodies or multi-transfusion because donor blood must be given whose antigens are negative for the patient's alloantibodies; it is important to prepare donor blood that has known variations of antigens so as not to react to the patient's alloantibodies. The Rhesus blood group system is the most important and clinically significant, so the initial phenotype examination recommended is for the Rhesus. The strategy is to start choosing a donor to be examined, preferably a donor who regularly donates

blood or a repeat donor. If the antigen is known, document it and record it as basic data to make it easier to find donor blood with certain antigen specifications. From these data, a list of permanent donors can be made for certain patients who already have alloantibodies so that they do not arise again or may not have aroused alloantibodies so that they can be given appropriate blood, the best transfusion, most appropriate to avoid transfusion reactions (Zhao et al., 2023).

Pre-transfusion examination at UDD PMI Depok City currently only tests for ABO and Rhesus D blood group antigens. C, c, E and e antigen tests are not systematically performed. Examining other Rh antigens (C, E, c and e) in pre-transfusion testing is useful in preventing alloimmunization, especially for multitransfusion patients. For this purpose, at UDD, PMI Depok City will try to start conducting checks on Rh C, E, c, and e antigens. Rh phenotype testing in blood donors in various countries, for example, in Karim et al.'s (2015) study in Pakistan, showed that most phenotypes were Dce/Dce (44%). The HDFN study in India found that out of 50 cases, as many as 22% were due to Rh mismatch (Baruah et al., 2022). India reported a case of HTR in a pregnant woman and multitransfusion patient due to anti-E (Asnawi et al., 2016). Transfusions that match the Rh phenotype can reduce the incidence of alloimmunization and the rate of adverse transfusion reactions for patients, especially in multi-transfusion patients (Sarkar et al., 2013). If a patient with a major Rh phenotype, such as Dce/Dce, receives a blood transfusion from a donor with a rare phenotype, such as DcE/DcE, there is a high possibility of a transfusion reaction (Dewi et al., 2019).

A regular blood donor is a blood donor who donates blood at least twice a year and has done so for at least two consecutive years. The study subjects were routine blood donors because routine blood donors had the most significant contribution to the number of blood bags transfused to patients.

Through phenotyping, the examination of the Rhesus system blood type in routine blood donors, especially blood type O at UDD PMI Depok City, is done in order to ensure patient safety by providing donor blood without antigens. Through this research, it is hoped that in the future, it can be used for safe blood transfusion by adjusting the Rh phenotype of the donor and patient.

## RESEARCH METHOD

This study was descriptive and observational with a cross-sectional design. The research subjects are regular blood donors at UDD PMI Depok City. This research was conducted on 140 samples from 140 routine blood donors with blood type O who had blood donated and met the inclusion criteria at UDD PMI Depok City from July to August 2023. Selection criteria are regular blood donors (after being certified by a doctor as healthy), donor blood samples that are not hemolyzed, negative antibody screening test results, and non-reactive Blood Transfusion Infection test results. The exclusion criteria were not 5% red blood cell suspension from Rh antigen testing, and the results were invalid; the DCT result was positive, and the donor sample came from the blood bag. This research material uses blood samples taken from venous blood of as much as 3 ml and then inserted into the EDTA tube for examination of Rh phenotype using a 5% erythrocyte suspension. The examination carried out is the Rh phenotype testing performed using C, c, E, and e antigens, according to the principle of antibody-antigen testing: hemagglutination by gel method.

## FINDINGS AND DISCUSSION

This study was conducted on 140 samples of routine blood type O blood donors who had donated blood and met the inclusion criteria at UDD PMI Depok City from July to August 2023. The characteristics of the study subjects, which include sex and age, are presented in Table 1.

**Table 1.** Sex and age distribution

No.	Variable	Sum	%
1.	Gender		
	Man	97	69,29
	Woman	43	30,71
2	Age Group		
	17-29	40	28,57
	30-59	98	70
	>60	2	1,43

In this study, there were more male donors than female donors. Subjects' ages ranged from 17 to 63 years, with a median age of 38. Similarly, [Sarkar et al.'s \(2013\)](#) research and [Gundrajukuppam et al. \(2016\)](#) ([Dewi et al., 2019](#)). From the results of the examination of 140 subjects, the frequency of the Rhesus antigen is presented in Figure 1.

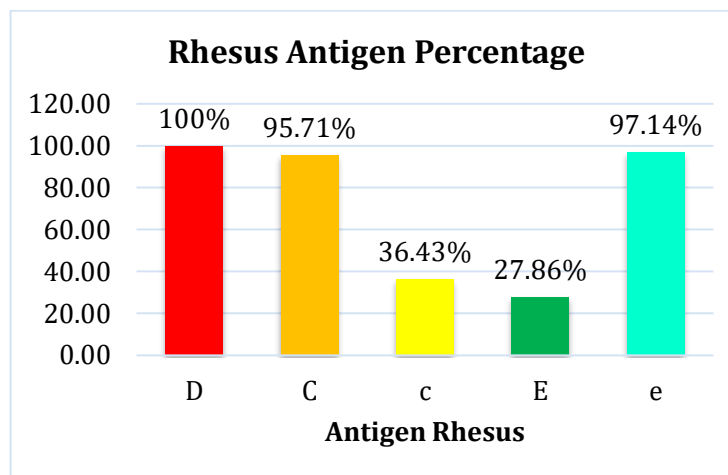
**Figure 1.** Rh antigen frequency diagram

Figure 1. shows the frequency of Rhesus system antigens in donor blood at UDD PMI Depok City. The e antigen is the second most common antigen found in blood donors, at 97.14%. Then, the next antigen that is often found is antigen C, as much as 95.71%, antigen c (36.43%), and the results of antigen E is the least antigen, namely (27.86%). The frequency of Rhesus antigens found in this study is similar to the study conducted by [Dewi et al. \(2019\)](#) in Bandung, which conducted Rhesus antigen tests from 142 donor samples, and all of them were Rhesus positive. The study results were obtained sequentially: antigen e as much as 98.6%, antigen C as much as 97.9%, antigen C as much as 38.7% and antigen E as much as 31.7% ([Dewi et al., 2019](#)). In research conducted by [Bogui et al. \(2014\)](#) in West Africa, it was found that the frequency of C and E antigens was higher, namely by 99% and 85%, respectively, while the frequency of C and E antigens was lower, respectively 21.97% and 13.82% ([Bogui et al., 2014](#)).

If erythrocytes express C and c and E and e antigens, then it can be assumed that the individual is heterozygous (Cc or Ee). However, if erythrocytes express only one of the antigens C or c and E or e, then the individual is assumed to be homozygous (CC/cc, EE/ee). Among Asians, the e antigen is the most common, followed by the C antigen, while the C antigen is more commonly found in blacks and Caucasians. Antigen E is the antigen with the least frequency found in all races ([Brecher, 2005](#)).

**Table 2.** Rhesus antigen frequency comparison

Research	% Rh Antigen					Number of Sample
	D	C	c	E	e	
This study (Indonesia)	100	95,71	36,43	27,86	97,14	140
Zhao et al. (2023) (India)	99,40	88,77	53,63	44,04	92,61	129.078
Baruah et al. (2022) (India)	99,05	92,38	51,43	20,95	97,14	315
Dewi et al. (2019) (Indonesia)	100	97,9	38,7	31,7	98,6	142
Gundrajukuppam et al. (2016) (India)	94,1	88	54,9	18,8	98,4	1000
Thakral et al. (2010) (India)	93,4	84,76	52,82	17,9	98,3	1.240
Garg et al. (2015) (India)	93,8	91,8	55,2	21,1	98,7	2.769
Karim et al. (2015) (Pakistan)	97	87	57	19	99	100

**Table 3.** Results of the Rh system antigen examination

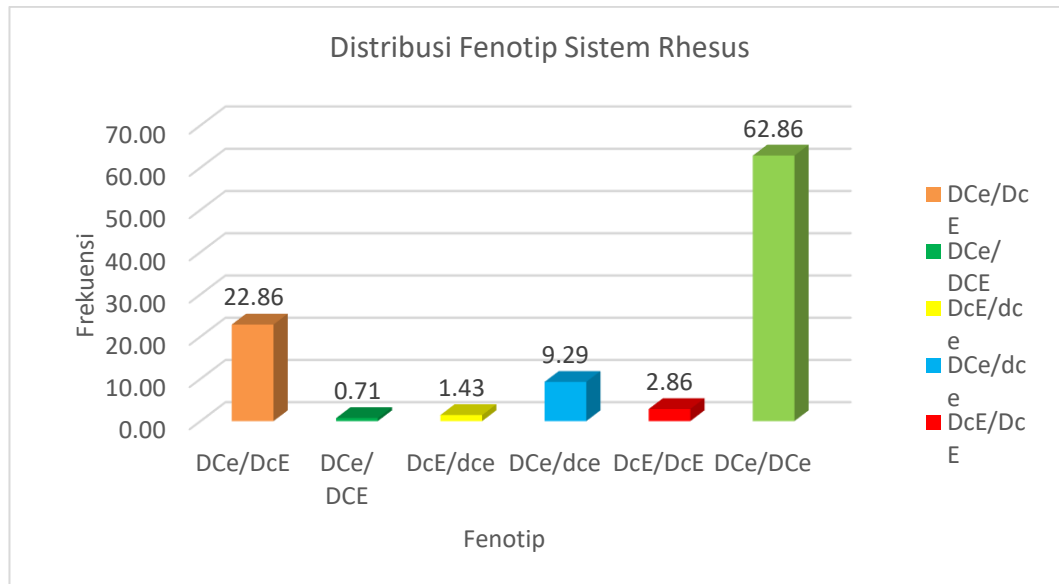
No.	Antigen	Number of Samples (n=140)	%
1.	D	140	100,00
2	C	134	95,71
3	c	51	36,43
4	E	39	27,86
5	e	136	97,14

Table 3 presents the results of Rhesus antigen testing on donor blood at UDD PMI Depok City. In the 140 subjects included in this study, D antigen was found in 100%. Other studies in which the majority of blood donors had D antigen were [Obimbo and Omanwa \(2023\)](#) (97,5%), [Garg et al. \(2015\)](#) (93.8%), [Karim et al. \(2015\)](#) (97%) and [Thakral et al. \(2010\)](#) (93,4%). [Zhao et al. \(2023\)](#) (99.40%). Similar results were also found in research conducted by [Gundrajukuppam et al. \(2016\)](#) on 1000 donors in India. Sequentially, the most common antigens found were E antigen as much as 98.4%, C antigen 88%, C antigen 54.9% and E antigen 18.8%. In research conducted by [Bogui et al. \(2014\)](#) in West Africa, frequency was found. C and E antigens are higher at 99% and 85%, respectively, while the frequency of C and E antigens is lower, respectively 21.97% and 13.82% ([Bogui et al., 2014](#)). Based on the results of the Rh antigen examination, then the Phenotype interpretation of Rh blood type is presented in Table 4.

**Table 4.** Results of Rhesus antigen examination and phenotypic interpretation

No	Antigen					Phenotype interpretation		Total (n=140)	
	D	C	c	E	e	Wiener	Fisher- Race	Sum	%
1	+	+	+	+	+	R <sub>1</sub> R <sub>2</sub>	DcE/DcE	32	22,86
2	+	+	-	+	+	R <sub>1</sub> R <sub>Z</sub>	DcE/DCE	1	0,71
3	+	-	+	+	+	R <sub>2</sub> r	DcE/dce	2	1,43
4	+	+	+	-	+	R <sub>1</sub> r	DcE/dce	13	9,29
5	+	-	+	+	-	R <sub>2</sub> R <sub>2</sub>	DcE/DcE	4	2,86
6	+	+	-	-	+	R <sub>1</sub> R <sub>1</sub>	DcE/DCE	88	62,86
Total								140	100

As a result of this study, six Rh blood system phenotypes were obtained. The most common phenotype found in the O Rh positive group sample in this study was DCCee (DcE/DcE) which was 62.86% (n = 88) and the rarest was DCCeE (DcE/DCE) as much as 0.71% (n = 1).



**Figure 2.** Rh phenotype distribution diagram in this study

**Table 5.** Comparison of phenotypic frequencies of Rhesus blood groups

Rhesus Phenotype Frequency	Present study		Musa et al		Garg et al. (2015)		Karim et al. (2015)		Thakral et al. (2010)		Dewi et al. (2019)	
	N= 140		N= 594		N= 2.769		N= 100		N= 1240		N= 142	
	n	%	n	%	n	%	n	%	n	%	n	%
DCe/DCe (R <sub>1</sub> R <sub>1</sub> )	88	62,86	330	56	1238	44,6	41	41	543	43,8	87	61,3
DCe/DcE (R <sub>1</sub> R <sub>2</sub> )	32	22,86	113	19	389	14	10	10	102	8,3	42	29,6
DcE/DcE (R <sub>2</sub> R <sub>2</sub> )	4	2,86	28	5	23	0,8	1	1	18	1,5	2	1,4
DCe/DCE (R <sub>1</sub> R <sub>Z</sub> )	1	0,71	14	2	-	-	-	-	-	-	-	-
DcE/DCE (R <sub>2</sub> R <sub>Z</sub> )	-	-	2	0,34	-	-	-	-	-	-	-	-
DCe/dce (R <sub>1</sub> r)	13	9,29	76	13	902	326	34	14	372	30	10	7,0
DcE/dce (R <sub>2</sub> r)	2	1,43	19	3	163	59	8	8	111	8,9	1	0,7
Dce/dce (R <sub>0</sub> r)	-	-	-	-	55	2	3	1	12	1	-	-
dce/dce (rr)	-	-	12	2	2	0,1	1	1	72	5,8	-	-
dCe/dCe (r'r')	-	-	-	-	-	-	1	1	7	0,5	-	-
dCe/dcE (r'r'')	-	-	-	-	-	-	1	1	3	0,2	-	-
<b>Total</b>	140		594		2.769		100		1240		142	

Table 5 shows a comparison of the Rhesus phenotype in this study with other studies. Variations in the phenotype of the Rhesus blood group in this study play a role in the frequency of alloimmunization and alloantibodies if the donor and patient phenotypes are different. Merizka's (2016) research said that the genotype and frequency of Rhesus C, c, E, and e antigens in Indonesia with a total of 86 samples of thalassemia recipients at RSCM found that the majority of people in Indonesia had a RHCE\*Ce genotype of 52 samples while the very rare genotypes were RHCE\*ce and RH\*cE which were only one sample. Examination of the Rh phenotypes of blood donors from different countries, for example, in a study conducted by Karim et al. (2015) in Pakistan, showed that the most common phenotypes were Dce/Dce (44%). Chitra's et al. (2021) study in India showed that the most common phenotypes were Dce/Dce (35%), rare phenotypes were dce/dce (7%), DcE/Dce (2%), and dCE/dce (2%). In the Khuzestan and Gilan study in Iran showed the prevalence of Rh antigens in antigens D (88.9%), C (74.1%), c (72.8%), E (30.9%), and e (96, 2%). Dawam et al., 2014, in his research, stated that the prevalence of alloimmunization was 5.64%, of which 52.17% was caused by Rhesus antibodies (anti-E 17%, anti-D 13%, and anti-C 13%). These Rhesus antibodies can cause incompatibilities in matching cross-tests, the occurrence of HDFN and HTR (Dhawan et al., 2014).

The "e" antigen and R1r phenotype (DCe/dce) are the most common (Bakhshandeh et al.,

2021). These results show similarities with studies conducted by researchers, which have the majority of Rhesus Ce antigen samples with the Rhesus antigen phenotyping method using the gel test method.

Phenotype examination is very useful namely, the time to prepare donor blood according to the patient's needs becomes shorter because there is no need for a match test examination, only need to see the patient's basic antigen data or alloantibody data owned by the patient and donor blood antigens through a computer that records as a data bank. Thus, the goal of improving patient safety is achieved. Patient and donor antigen data are documented. The patient is given the right donor blood, which is to get donor blood that is the same antigen as the patient, or if the patient already has alloantibodies, donor blood that is antigen-negative for existing antibodies can be found. The patient avoids transfusion reactions; donor blood does not lyse quickly, and the frequency of transfusions becomes less frequent.

## CONCLUSIONS

The frequency of phenotype variation of the Rhesus system blood group in routine blood donations at UDD PMI Depok City is sorted from the most are R<sub>1</sub>R<sub>1</sub> (DCe/DCe) 62,86% (n=88), R<sub>1</sub>R<sub>2</sub> (DCe/DcE) 22,86% (n=32), R<sub>1</sub>r (DCe/dce) 9,29% (n=13), R<sub>2</sub>R<sub>2</sub> (DcE/DcE) 2,86% (n=4), R<sub>2</sub>r (DcE/dce) 1,43% (n=2), and R<sub>1</sub>R<sub>2</sub> (DCe/DCE) 0,71% (n=1).

The study was limited to only 140 donor samples due to cost constraints, so we used extended antigen typing; if the number was greater, it was likely that more differences would be found in the rhesus blood group phenotype. If possible, this study will continue with the erythrocyte antigen genotyping study at the molecular level in donors and patients.

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