Case report

Case report of a Thai blood donor with weak expression of RHCE antigens

Siwaporn Namdang[1], Ploymanee Suwanwootichai[1], Morakot Emthip[1], Sirilak Phiancharoen[1], Aphisit Thongthaisin[1], Sasitorn Bejrachandra[1], Yew-Wah Liew[2] and Vashiraporn Yonwises[3]

[1]National Blood Centre, Thai Red Cross Society; [2]Red Cell Reference Laboratory, Clinical Service and Research, Australian Red Cross Lifeblood, Australia; [3]Regional Blood Centre VIII Nakhonsawan Province, Thai Red Cross Society

Abstract:

The Rh blood group is encoded by the RHCE gene, including C, E, c, and e, which are clinically important in causing hemolytic transfusion reactions (HTRs) and hemolytic disease of the fetus and newborn (HDFN). In this report, we present a case of weak expression of C, E, c, and e antigens in a Thai blood donor. The donor's RHCE antigens were tested using standard serological methods with monoclonal antibodies from two different clones using the column agglutination test (CAT) method by Ortho Vision Max analyzer. The blood donor result using the first clone of monoclonal antibodies was C-E-c-e- and using the second clone of monoclonal antibodies was C+E-c-e-. Differences between the two monoclonal antibody clones' results were observed, therefore using other serological techniques, adsorption-elution and titration, and the molecular technique, next-generation sequencing (NGS) combined with genetic inheritance studies, to be able to confirm RHCE antigens results. It was found that the blood donor and her parents had a hybrid of RHD and RHCE genes, which the predicted hybrid is possibly RHCE(1-2)-D(3-7)-CE(8-10) or RHCE(1)-D(2-8)-CE(9-10) or RHCE(1-2)-D(3-8)-CE(9-10) or RHCE(1)-D(2-7)-CE(8-10). This may be causing reduced or lack of expression of some or all of the RHCE antigens. The predicted phenotype of the blood donor and her parents was C+E-c-e+. Due to this testing cannot definitively identify the variant's location, further testing using long-read sequencing is required to confirm the position of the hybrid gene.

Keywords : ● *RHCE blood group* ● *Adsorption-elution* ● *Titration* ● *Next-generation sequencing*

J Hematol Transfus Med. 2025;35:65-72.

Received 9 January 2025 Corrected 17 February 2025 Accepted 4 March 2025

Correspondence should be addressed to Siwaporn Namdang, National Blood Centre, Thai Red Cross Society, 1871 Henri Dunant Rd, Pathumwan, Bangkok 10330 Tel: 02-2639600-99 E-mail: siwaporn.na@redcross.or.th

รายงานผู้ป่วย

รายงานผู้บริจาคโลหิตไทยที่มี weak expression of RHCE antigens

ศิวาพร นามแดง[1] พลอยมณี สุวรรณวุฒิชัย[1] มรกต เอมทิพย์[1] ศิริลักษณ์ เพียรเจริญ[1] อภิสิทธิ์ ทองไทยสิน[1]
ศศิธร เพชรจันทร[1] Yew-Wah Liew[2] และ วชิราภรณ์ ยนต์วิเศษ[3]
[1]ศูนย์ห้องปฏิบัติการอ้างอิง ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย [2]Red Cell Reference Laboratory, Clinical Service and Research, Australian Red Cross Lifeblood, Australia [3]ภาคบริการโลหิตแห่งชาติที่ 8 จังหวัดนครสวรรค์ สภากาชาดไทย

บทคัดย่อ

หมู่โลหิตระบบ Rh ที่เกิดจากยีน RHCE คือแอนติเจน C, E, c และ e มีความสำคัญทางคลินิกในการทำให้เกิด hemolytic transfusion reaction (HTRs) และ hemolytic disease of the fetus and newborn (HDFN) รายงานนี้เป็นการศึกษาการแสดงออกของแอนติเจน C, E, c และ e ที่ผิดปกติในผู้บริจาคโลหิต 1 ราย โดยตรวจแอนติเจน RHCE ในผู้บริจาคโลหิตด้วยเทคนิคการตรวจทางซีโรโลยีที่เป็นมาตรฐานในงานธนาคารเลือด ด้วยน้ำยา monoclonal antibody ที่แตกต่างกัน 2 clones ด้วยวิธี column agglutination test (CAT) โดยใช้เครื่องวิเคราะห์อัตโนมัติ Ortho Vision Max ผลการตรวจแอนติเจนในผู้บริจาคโลหิตพบว่าน้ำยา clone ที่ 1 ได้ผลเป็น C-E-c-e- และ clone ที่ 2 ได้ผลเป็น C+E-c-e- ซึ่งผลของน้ำยา monoclonal 2 clones ขัดแย้งกัน ดังนั้น จึงใช้เทคนิคการตรวจทางซีโรโลยี อื่นๆ คือ adsorption-elution และ titration และเทคนิคด้านอณูชีววิทยา คือ next-generation sequencing ร่วมกับการศึกษาการถ่ายทอดทางพันธุกรรมร่วมด้วย จึงสามารถสรุปผลแอนติเจนของผู้บริจาคได้ถูกต้อง ผลการตรวจทางด้านอณูชีววิทยาพบว่า ผู้บริจาคโลหิต บิดาและมารดา มียีนลูกผสมของ RHD และ RHCE genes ที่เป็นไปได้คือ RHCE(1-2)-D(3-7)-CE(8-10) หรือ RHCE(1)-D(2-8)-CE(9-10) หรือ RHCE(1-2)-D(3-8)-CE(9-10) หรือ RHCE(1)-D(2-7)-CE(8-10) ซึ่งน่าจะเป็นสาเหตุของการลดลงของการแสดงออกของแอนติเจน RHCE โดยความน่าจะเป็นของแอนติเจนของผู้บริจาคโลหิต บิดาและมารดาคือ C+E-c-e+ แต่ผลการทดสอบไม่สามารถระบุตำแหน่งของ variant ได้อย่างแน่ชัด ต้องทำการทดสอบเพิ่มเติมโดยเทคนิค long read sequencing เพื่อยืนยันตำแหน่งของยีนลูกผสมต่อไป

คำสำคัญ : ● *หมู่โลหิตระบบ Rh(CE)* ● *Adsorption-elution* ● *การไทเทรต* ● *Next-generation sequencing*
วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2568;35:65-72.

Introduction

The Rh blood group system is one of the most complex blood groups known in humans[1-3], with variations due to deletions, gene conversions, and missense mutations[4], and is the second-most clinically significant blood group to ABO.[5-7] The Rh blood group system encompasses two highly homologous genes, *RHD* and *RHCE*,[3,5,7-8] closely located on the short arm of chromosome 1 (1p36.11)[7,9-11], which encode RhD and RhCE proteins differing in only 32-35 of 417 amino acids.[7] The *RHCE* gene encodes the RhCE protein, carrying C (RH2) or c (RH4) and E (RH3) or e (RH5) antigens.[4,7] Common RhCE antigens are highly immunogenic, and their alloantibodies have been involved in hemolytic transfusion reactions (HTRs)[7] and hemolytic disease of the fetus and newborn (HDFN) which IgG antibodies passed through the mother's placenta to the fetus and destroy the baby's red blood cells[12]. All patients require compatible red blood cell (RBC) components for safety. In this case, we will confirm RHCE antigen in blood donors according to standard of Food and Drug Administration, United States (U.S.- FDA), red blood cell antigen tests other than ABO and Rh(D) should be tested with at least 2 different monoclonal clones which consistent results. Differences between the two monoclonal antibody clones' results were observed, therefore using other serological techniques such as adsorption-elution[13] to detect weak expression of RHCE antigens and titration for measuring the level of antigen expression, and the molecular technique is next-generation sequencing (NGS)[14] for analyzing the changed base sequence in deoxyribonucleic acid (DNA), including the study of genetic inheritance to confirm RHCE antigens.

Case presentation

A case of a 33-year-old Thai female blood donor, 4th blood donation from Regional Blood Centre 8th Nakhonsawan Province, Thai Red Cross Society, was tested for RHCE antigens. C, E, c and e phenotyping was performed using the column agglutination test (CAT) method by the Ortho Clinical Diagnostic, Switzerland on Ortho Vision Max analyzer. Blood samples from the donor, along with her parents blood samples, were sent to the Reference Laboratory Centre at the National Blood Centre (NBC), Thai Red Cross Society for confirmed C, E, c and e phenotyping. The donor's RHCE antigens were tested using standard serological methods with monoclonal antibodies from two different clones, as detailed in Table 1.

The blood donor results showed that the first clone of monoclonal antibodies was C-E-c-e- and the second clone of monoclonal antibodies was C+E-c-e- (Table 1). Differences between the two monoclonal antibody clones' results were observed. Her parents blood samples were tested for phenotyping which the results showed C+E-c-e+ (Table 1). According to Mendel's laws of inheritance, the expected phenotype of the blood donor would be C+E-c-e+, which is consistent with her parents. Based on the hypothesis, it is likely that the blood donor has antigens similar to those of her parents, but with very weak expression. We have performed other serological techniques to confirm the C, E, c, and e phenotype results.

First of all, we performed adsorption-elution test. The red blood cells of the blood donor were adsorbed with anti-C and anti-e, incubated at 37°C for 60 minutes at the appropriate temperature for Rh antibodies.

Table 1 The results of C, E, c, and e phenotype using monoclonal antibodies from two different clones in the blood donor, and her parents

Serology	Anti-C clone		Anti-E clone		Ant-c clone		Anti-e clone	
	1. MS 24	2. MS 273	1. DEM 1	2. MS 80/258	1. H 48	2. 951	1. MS 16/21/63	2. MS 62
Donor	0	2+	0	0	0	0	0	0
Father	4+	4+	0	0	0	0	4+	4+
Mother	4+	4+	0	0	0	0	4+	4+

Table 2 The results of the titration tests for anti-C and anti-e with red blood cells from the donor's parents

Cells	Anti-sera	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1,024	1:2,048	The strength of antigen expression (Titer)
Father	C	4+	4+	3+	1+	1+	1+	W	0	0	0	0	0	32
Mother	C	4+	4+	3+	3+	1+	1+	W	W	0	0	0	0	32
1. normal C+E-c-e+ cell	C	4+	4+	4+	4+	3+	2+	1+	1+	W	0	0	0	128
2. normal C+E-c-e+ cell	C	4+	4+	4+	4+	3+	3+	2+	1+	W	0	0	0	128
Father	e	4+	4+	4+	3+	3+	2+	1+	1+	0	0	0	0	128
Mother	e	4+	4+	4+	4+	4+	4+	3+	1+	1+	0	0	0	256
1. normal C+E-c-e+ cell	e	4+	4+	4+	4+	4+	4+	2+	1+	1+	W	0	0	256
2. normal C+E-c-e+ cell	e	4+	4+	4+	4+	4+	3+	3+	2+	1+	0	0	0	256

- The agglutination reaction was graded, with a final titer being determined as 1+, representing the highest strength of the antigen (titer)
- The gray color indicates the final 1+ agglutination reaction, representing the highest strength of the antigen (titer)

Table 3 The scoring system to calculate the strength of the antigen.

Cells	Anti-sera	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1,024	1:2,048	Total score
Father	C	12	12	10	5	5	5	2	0	0	0	0	0	51
Mother	C	12	12	10	10	5	5	2	2	0	0	0	0	58
1. normal C+E-c-e+ cell	C	12	12	12	12	10	8	5	5	2	0	0	0	78
2. normal C+E-c-e+ cell	C	12	12	12	12	10	10	8	5	2	0	0	0	83
Father	e	12	12	12	10	10	8	5	5	0	0	0	0	74
Mother	e	12	12	12	12	12	12	10	5	5	0	0	0	92
1. normal C+E-c-e+ cell	e	12	12	12	12	12	12	8	5	5	2	0	0	92
2. normal C+E-c-e+ cell	e	12	12	12	12	12	10	10	8	5	0	0	0	93

- Scores differing by more than 10 points compared to normal cells are clinically significant.[11]
- The gray color indicates the scores are considered clinically significant.

After that acid-elution was performed, and the eluate was tested with C+c- and e+E- cells to detect anti-C and anti-e that could bind to the weak antigens on the cell surface. The testing revealed that the eluate contained both anti-C and anti-e. From these results, the phenotype of C+E-c-e+, but a weak expression for C and e antigens were observed. We suspect that the donor's parents had an abnormal strength of antigen expression for C and e. In addition, we use titration to test the strength of the antigen with the red blood cells of the donor's parents, as detailed in Table 2. The results of the titration tests for anti-C and anti-e by grading the agglutination reaction was graded as 0, 1+, 2+, 3+, and 4+. The strengths of the antigen results of the donor's parent cell were compared with normal C+E-c-e+ cells. The agglutination reaction was graded, with a final titer being determined as 1+, representing the highest strength of the antigen (titer). The titer of the C antigen was 32 in the father and mother, compared to 128 in normal cells. The titer of the e antigen was 128 in the father and 256 in the mother, compared to 256 in normal cells. In cases where the difference in antigen titer exceeds 1 two-fold dilution, it is considered clinically significant.[11] The results showed that only antigen C was significantly weakly expressed in the father and mother. The scoring system for the evaluation of quantitative antigen expression levels uses the definition of the Association for the Advancement of Blood & Biotherapies (AABB) standards for Blood Banks and Transfusion Services, the total scores for each result are shown in Table 3. The scores are recorded as follows: 4+ = 12 points; 3+ = 10 points; 2+ = 8 points; 1+ = 5 points; and weak = 2 points.[11]

The score of the C antigen was 51 points in the father, and 58 points in the mother, compared to 78 and 71 points in normal cells, respectively. The score of the e antigen was 74 points in the father, and 92 points in the mother, compared to 92 and 93 points

in normal cells, respectively. When comparing the differences in scores, it was found that the father had a lower strength of C antigen expression than normal cells, with differences of 27 and 20 points, respectively. In addition, the father had a lower strength of e antigen expression than normal cells, with differences of 18 and 19 points, respectively. Meanwhile, the mother had a lower strength of C antigen expression than normal cells, with differences of 20 and 13 points, respectively. In addition, the mother had a lower strength of e antigen expression than normal cells, with differences of 0 and 1 point, respectively. Scores differing by more than 10 points are considered clinically significant.[11] In summary, the scoring results indicate that the father has a lower strength of C and e antigen expression, while the mother has only a lower strength of C antigen expression. When comparing the results of the strength antigen expression between grading and scoring, it is clear that there are differences in the indication of the clinical significance of the strength of antigen expression. The scoring system provides a clearer and more quantitative assessment of abnormalities, whereas grading offers a qualitative assessment. The scoring system helps to highlight significant differences in the strength of antigen expression more effectively.

Finally, to investigate base sequence variations and genetic inheritance within her family. The blood samples from the donor and her parents were sent to the Australian Red Cross Lifeblood for molecular analysis utilizing next generation sequencing (NGS). It was found that the blood donor and her parents had a hybrid of *RHD* and *RHCE* genes, which the predicted hybrid is possibly *RHCE(1-2)-D(3-7)-CE(8-10)* or *RHCE(1)-D(2-8)-CE(9-10)* or *RHCE(1-2)-D(3-8)-CE(9-10)* or *RHCE(1)-D(2-7)-CE(8-10)*. This may be causing reduced or lack of expression of some or all of the RHCE antigens. Her parent is that the probable genotype is *RHD*01/01* and *RHCE*02* together with one of the previous hybrids with a probable phenotype of C+E-c-e+. The predicted phenotype of the donor parents was C+E-c-e+, which matched the antigens detected using serological techniques. Since the father showed weak expression of the C and e antigens and the mother showed only weak expression of the C antigens, it is hypothesized that the hybrid gene may still retain exon 2 of the *RHCE* gene, as exon 2 plays a major role in the expression of C antigen. In addition, the blood donor's probable genotype is *RHD*01/01* and homozygous for either of the previous hybrids. Due to this testing cannot definitively identify the variant's location, further testing using long-read sequencing is required to confirm the position of the hybrid gene.

Materials and Methods

Study samples

Three samples (father, mother, and donor) were investigated in this study. RHCE antigens phenotyping, adsorption-elution, and titration studies were performed at the National Blood Centre (NBC), Thai Red Cross Society. Genotyping by DNA sequencing was performed at the Australian Red Cross Lifeblood. This study has ethics approval from the NBC, Thai Red Cross Society Ethics Committee (NBC 4/2025).

Hemagglutination tests

Standard hemagglutination tests were performed using column agglutination technology (Reverse, Ortho clinical diagnostic, Switzerland) following the manufacturer's recommendation. RHCE phenotyping was tested with monoclonal antibodies from two different clones and was used according to the manufacturer's instructions. The first clone of monoclonal antibodies included Anti-C clone MS 24 (Merck Millipore), anti-E clone DEM1 (NBC), anti-c clone H48 (NBC), and anti-e clone MS 16/21/63 (Merck Millipore). The second clone of monoclonal antibodies included anti-C clone MS 273 (Merck Millipore), anti-E clone MS 80/258 (Merck Millipore), anti-c clone 951 (Merck Millipore), and anti-e clone MS 62 (Merck Millipore).

Adsorption-elution

The red blood cells of the blood donor were adsorbed with anti-C and anti-e, incubated at 37°C for 60 minutes at the appropriate temperature for Rh antibodies. Acid-elution (DiaCidel Reagents kit, Bio-Rad) was tested according to the manufacturer's instructions.

Titration

First, diluted anti-C and anti-e with 5% bovine serum albumin in a serial two-fold dilution starting from 1:2 to 1:2,048, pipetted 200 μL per each dilution, added 50 μL of 3-5% red cell suspension from the donor's parent, incubated at 37°C for 60 minutes, washed 3 times with 0.9% NSS, add two drops anti-human globulin, centrifuged at 1,000 g for 15 seconds, last, read the agglutination reaction.

DNA sequencing

DNA extraction equipment (QIAsymphony, QIAGEN) was used to extract DNA from EDTA-whole blood samples as recommended by the manufacturer. Massively parallel sequencing (MPS) was performed using a custom-designed panel (Illumina, San Diego, USA).[15] DNA were prepared using the Illumina DNA prep with an enrichment kit. DNA sequencing was performed on a DNA sequencer (Illumina MiSeq) as per the manufacturer's instructions. Binary alignment map (BAM) files were generated after the reads were aligned to the human reference genome GRCh37/Hg19 on the MiSeq. BAM files were then imported into bioinformatics analysis software (CLC genomics workbench software 20, QIAGEN) to generate annotated variant call files as previously described.[15]

Results

RBC Phenotyping

The donor and her parents' RHCE phenotyping was tested using standard serological methods with monoclonal antibodies from two different clones, as detailed in Table 1.

Adsorption-Elution

The donor sample was tested by adsorption-elution test. The testing revealed that the eluate contained both anti-C and anti-e. Results indicated the donor had C+E-c-e+, but a weak expression for C and e antigens.

Titration

The strengths of the antigens with the red blood cells of the donor's parents are detailed in Table 2&3.

DNA sequencing

The blood donor and her parents had a hybrid form of *RHD* and *RHCE* genes**,** as detailed in Table 4&5.

Table 4 The DNA sequencing result of the donor

Gene variation	Interpretation
RHD&RHCE (RH) CNVs	Normalised mean coverage ratios for *RHD* appears to indicate the presence of two copies of the *RHD* gene noting also an apparent overrepresentation when compared to the *RHCE* gene. Also see below.
RHCE (RH) CNVs	Normalised mean coverage rations for *RHCE* initially indicates two copies of the *RHCE*02* allele however subsequent interrogation of the sequence read mapping for *RHCE* incicates it is more likely a single copy with the second allele being the same suspected hybrid allele that is present in his daughter's sample.
	The predicted hybrid is possibly *RHCE(1-2)-D(3-7)-CE(8-10)* or *RHCE(1)-D(2-8)-CE(9-10)* or *RHCE(1-2)-D(3-8)-CE(9-10)* or *RHCE(1)-D(2-7)-CE(8-10)*. This may be causing reduced or lack of expression of some or all of the *RHCE* antigen. (Please note that due to sequence homology we cannot determine whether exons 2 and 8 in the hybrid variants are *RHD* or *RHCE* derived) The hybrid alleles *RH(1)-D(2-8)-CE(9-10)* and *RHCE(1)-D(2-7)-CE(8-10)* have been previously reported 3 and may be present in this sample.
	Our interim outcome is that the probable genotype is *RHD*01/01* and *RHCE*02* together with one of the previous hybrids.

Table 5 The DNA sequencing result of the donor's parents. The donor's parents had the same result.

Gene variation	Interpretation
RHD (RH) CNVs	Normalised mean coverage ratios for *RHD* appears to indicate the presence of two copies of the *RHD* gene noting also an apparent overrepresentation when compared to the *RHCE* gene. Also see below.
RHCE (RH) CNVs	Normalised mean coverage ratio for *RHCE* initially indicates two copies of the *RHCE*02* allele however subsequent interrogation of the sequence read mapping for *RHCE* incidates a hybrid allele is present at homozygous level.
	The predicted hybrid is possibly *RHCE(1-2)-D(3-7)-CE(8-10)* or *RHCE(1)-D(2-8)-CE(9-10)* or *RHCE(1-2)-D(3-8)-CE(9-10)* or *RHCE(1)-D(2-7)-CE(8-10)*. This may be causing reduced or lack of expression of some or all of the *RHCE* antigen. (Please note that due to sequence homology we cannot determine whether exons 2 and 8 in the hybrid variants are *RHD* or *RHCE* derived) The hybrid alleles *RH(1)-D(2-8)-CE(9-10)* and *RHCE(1)-D(2-7)-CE(8-10)* have been previously reported 3 and may be present in this sample.
	Our interim outcome is that the probable genotype is *RHD*01/01* and homozygous for either of the previous hybrids.

Discussion

In this case report, differences between the two monoclonal antibody clones' results were observed, so other serological techniques were used, including adsorption-elution and titration. Moreover, by comparing the results of the strength antigen expression between grading and scoring, the scoring system helps to highlight significant differences in the strength of antigen expression more effectively. The molecular technique was next-generation sequencing (NGS) to investigate base sequence variations and genetic inheritance. The study of genetic inheritance is very helpful in investigating the probability of RHCE antigens.

Conclusion

The results showed that the blood donor had the probable phenotype C+E-c-e+ with weak C and e antigen expression. The blood donor and her parents had a hybrid of *RHD* and *RHCE* genes. This may be causing reduced or lack of expression of some or all of the RHCE antigens.

References

1. *Avent ND, Reid ME. The Rh blood group system: a review. Blood. 2000;95(Suppl 2):S375-87.*
2. *Agre P, Cartron JP. Molecular biology of the Rh antigens. Blood. 1991;78(Suppl 3):S551-63.*
3. *Daniels G. Human blood groups. Chichester: Wiley-Blackwell; 2013.*
4. *Mouro I, Colin Y, Cherif-Zahar B, Cartron JP, Le Van Kim C. Molecular genetic basis of the human Rhesus blood group system. Nature Genetics. 1993;5:62-5.*
5. *Dean L. Blood groups and red cell antigens. Bethesda, MD: National Center for Biotechnology Information (US); 2005.*
6. *Flegel WA, Wagner FF. Molecular genetics of RH. Vox Sang. 2000;78(Suppl 2):109-15.*
7. *Sippert E, Arnoni CP, Rios M. Impact of RHCE variability and complexity in transfusion medicine: a narrative review. Ann Blood. 2023;8:8. doi: 10.21037/aob-21-76.*
8. *Wagner FF, Flegel WA. Review: the molecular basis of the Rh blood group phenotypes. Immunohematology. 2004;20(Suppl 1):S23-36.*
9. *Westhoff CM. The Rh blood group system in review: a new face for the next decade. Transfusion. 2004;44(Suppl 11):S1663-73.*
10. *Chérif-Zahar B, Mattéi MG, Le Van Kim C, Bailly P, Cartron JP, Colin Y. Localization of the human Rh blood group gene structure to chromosome region 1p34.3-1p36.1 by in situ hybridization. Human Genetics. 1991;86(Suppl 4):398-400.*

11. *Cohn CS, Delaney M, Johnson ST, Katz LM, Schwartz J, editor. Technical Manual. 21st ed. Bethesda, MD: American Association of Blood Banks; 2023.*
12. *Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen: FactsBook. 3rd ed. Boston: Academic Press; 2012.*
13. *Johnson ST, Judd WJ, Storry JR, editors. Judd's methods in immunohematology. 4th ed. Basel: S Karger AG; 2008.*
14. *Anderson MW, Schrijver I. Next generation DNA sequencing and the future of genomic medicine. Genes (Basel). 2010;1(Suppl 1):38-69.*
15. *Schoeman EM, Lopez GH, McGowan EC, Millard GM, O'Brien H, Roulis EV, et al. Evaluation of targeted exome sequencing for 28 protein-based blood group systems, including the homologous gene systems, for blood group genotyping. Transfusion. 2017;57:1078-88.*