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CASE REPORT

TRANSFUSION

# Transfusion management and hemoglobin-based oxygen carrier treatment in a patient with anti-Rh17 antibody

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## Abstract

**Background:** A 54-year-old Hispanic OPos female with known history of anti-Rh17 antibodies was diagnosed with Philadelphia-Chromosome positive (Ph+) acute lymphoblastic leukemia (ALL). Rh17, also known as $Hr_0$, is a high-frequency antigen composed of several epitopes on the RhCE protein. Anti-Rh17 antibodies can be made by individuals with missing or varied C/c, E/e antigens. Anti-Rh17 antibodies are clinically significant given multiple case reports of hemolytic disease of the fetus and newborn (HDFN). Finding compatible units for patients with anti-Rh17 can be particularly difficult given that only 1 in 100,000 people are Rh17 negative.

**Study Design and Methods:** Search for compatible units was conducted by the American Rare Donor Program (ARDP) with no leads. After chemotherapy induction and despite erythropoiesis stimulating agent administration, the patient's hemoglobin continued to trend down to a nadir of 2.8 g/dL. Here we report transfusion of incompatible pRBC to this patient with critically symptomatic anemia. HBOC-201 (Hemopure) was obtained and administered under an emergency compassionate/expanded access designation from the Food and Drug Administration (FDA) under an emergency Investigational New Drug (IND) application.

**Results and Discussion:** Overall difficulties in this case included the challenge of finding compatible units, dilemma of transfusing incompatible units in a patient with severe anemia and obtaining alternatives to blood products. This case report demonstrates the successful use of HBOC-21 in treating life-threatening anemia.

**List of Abbreviations:** ALB, Albumin; AGT, Antiglobulin test; ARC, American red cross; ALL, Acute lymphoblastic leukemia; DAT, Direct antiglobulin test; eIND, Expanded access use; ESA, Erythropoiesis stimulating agent; FDA, Food and drug administration; HDFN, Hemolytic disease of the fetus and newborn; HBOC, Hemoglobin-based oxygen carriers; IS, Immediate spin; IAT, Indirect antiglobulin test; IND, Investigational new drug; ICU, Intensive care unit; IVIG, Intravenous immune globulin; LDH, Lactate dehydrogenase; MMA, Monocyte monolayer assay; NHSN, National healthcare safety network; PEG, Polyethylene glycol; Rh, Rhesus blood group system; RBC, Red blood cell; SNP, Single nucleotide polymorphism.

**KEYWORDS**

anti-Hr0, anti-Rh17, hemoglobin-based oxygen carriers , RBC antibody, RBC transfusion, transfusion practices (adult)

## 1 | INTRODUCTION

The Rh system is a highly immunogenic, complex, and polymorphic blood group system with 56 currently characterized Rh antigens.[1] Antigens are located on two proteins, RhD and RhCE, encoded by two genes, *RHD* and *RHCE*, that are closely linked on chromosome 1 (1p36.11).[2] Rh17, also known as $Hr_0$, is a high-frequency antigen, with a prevalence of almost 100%, composed of several epitopes on the RhCE protein.[3] Anti-Rh17 antibodies can be made by individuals with missing or varied C/c, E/e antigens such as the phenotypes: D- -, D··, Dc-, $DC^W$-.[4] Finding compatible blood for patients with anti-Rh17 can be particularly difficult given that less than 1 in 100,000 people are Rh17 negative.[5] The anti-Rh17 antibody has been reported in a variety of populations, and there have been case reports of hemolytic disease of the fetus and newborn (HDFN) ranging from mild to severe.[4,6,7] Alternatives to blood transfusion including hemoglobin-based oxygen carriers (HBOCs) such as HBOC-201 (hemoglobin glutamer-250 [bovine]; "Hemopure," HbO2 Therapeutics LLC, Souderton, PA) have been developed to treat patients with severe anemia in which transfusion is not an option.[8] However, despite approval for use in countries such as Russia and South Africa, HBOC-201 in the United States is only available through the FDA's expanded access program.[9] HBOC-201 is made by the polymerization of bovine hemoglobin to prevent degradation.[10] Positive outcomes with HBOCs have been reported in various causes of anemia.[11] Specifically, use of HBOC-201 has been shown to safely mitigate transfusion when patients were treated with up to 10 units of HBOC-201.[12] However, there are several known and reported adverse effects of HBOCs including risk of myocardial infarction, gastrointestinal symptoms, transient methemoglobinemia, intravascular volume overload, and hypertension (including pulmonary hypertension).[11,13,14]

To our knowledge, no prior case reports have been published on the transfusion of incompatible blood to non-HDFN patients with anti-Rh17 antibodies. Additionally, this case report demonstrates the successful use of HBOC-201 in treating life-threatening anemia.

## 2 | CASE PRESENTATION

A 54-year-old Hispanic OPos female with known history of anti-Rh17 antibodies was transferred to an academic hospital for management of a new diagnosis of Philadelphia-chromosome-positive acute lymphoblastic leukemia (Ph + ALL). Anti-Rh17 antibodies were first identified by the American Red Cross (ARC) 5 years prior. At that time, she had no reported history of transfusion and had five previous pregnancies in Mexico that were associated with elevated bilirubin and severe jaundice. The patient's blood group was O, RhD+ without discrepancy. Direct antiglobulin test (DAT) and auto control (AC) were negative while the antibody screening panel was strongly positive with all unmodified and chemically treated cells tested. The only donor cells that did not react at polyethylene glycol- indirect antiglobulin test (PEG-IAT) were D-- phenotype cells. Anti-$Hr_0$ was identified and demonstrated reactivity at room temperature, 37°C-albumin, albumin-IgG-antiglobulin test, and PEG-IgG-antiglobulin test. Antibodies to other significant antigens were ruled out via adsorption. Molecular results from SNP testing showed low signal for the *RHCE* markers 676 G > C, 773 G > C, and 1006 G > T, consistent with variant or null alleles within the RHCE gene. Autologous donations were collected at this time.

**TABLE 1** Blood bank pre-transfusion testing.

| Test | Method | Result |
|---|---|---|
| ABO | Gel | O |
| RhD | Gel | Positive |
| Antibody screening with 3 Cell panel | Gel, Tube (PEG, LISS, DTT treated, unenhanced) | 4+ agglutination in all cells |
| Auto control | Gel, Tube (PEG, LISS, DTT treated, unenhanced) | Negative |
| Direct antiglobulin test | Tube | Negative |
| Antibody identification, 11 cell panel | Gel | 4+ agglutination in all cells |
| Cross match with random donors | Tube, LISS, IAT | Incompatible with all donors (4+ in all systems) |

Abbreviations: DTT, dithiothreitol; LISS, low ionic strength saline; PEG, polyethylene glycol.

**TABLE 2** Pre- and posttransfusion testing results.

| Test | Pre-transfusion | Posttransfusion | Reference range |
|---|---|---|---|
| Haptoglobin | – | 160 mg/dL | 51–192 mg/dL |
| Lactic dehydrogenase | – | 226 U/L | 116–245 U/L |
| Total bilirubin | 0.4 mg/dL | 1.4 mg/dL | 0.1–1.0 mg/dL |
| Unconjugated bilirubin | 0.2 mg/dL | 1.1 mg/dL | 0.1–1.1 mg/dL |
| Conjugated bilirubin | 0.2 mg/dL | 0.4 mg/dL | 0.0–0.3 mg/dL |
| Hemoglobin | 2.8 g/dL | 2.5 g/dL | 11.5–15.5 g/dL |
| Direct antiglobulin test | Negative[a] | Negative[a] | Negative |
| Visual evidence of hemoglobinemia | Negative | Negative | Negative |

[a]Negative with IgG and C3.

At the outside hospital prior to transfer, the patient received two units of allogeneic Rh-17 negative pRBCs without complication. The units were identified through local blood suppliers and the American Rare Blood Donor Program (ARDP). Three of the patient's children as well as the patient's half sibling were tested for directed donation, however, were all found to be positive for the C, E, c or e antigens. Upon transfer and admission, the patient's hemoglobin was 7.1 g/dL, at which time she was stable and asymptomatic. In anticipation for future transfusion, blood was requested from the ARDP. Blood bank workup revealed a positive antibody screen with 4+ pan-reactivity in gel and tube testing (polyethylene glycol, low ionic strength saline, unenhanced, and dithiothreitol treated). DAT and AC were negative (Table 1). The referring hospital provided reference laboratory adsorption results which showed the historical anti-Rh17 antibodies and no other clinically significant alloantibodies. Patient genotyping and Monocyte monolayer assay (MMA) samples were sent to the ARC reference laboratory. Given the patient's imminent need for blood transfusion, approval for HBOC-201, a hemoglobin-based oxygen carrier, was submitted to the FDA at this time for Expanded Access use.

The patient was initiated on dasatinib and corticosteroids for treatment of her Ph + ALL as part of clinical trial NCT04747912. Following initiation of treatment and despite daily erythropoiesis stimulating agent (ESA) administration, hemoglobin decreased to 2.8 g/dL. The patient was transferred to the medical intensive care unit for closer monitoring with continuous telemetry and pulse oximetry; the patient was also placed on oxygen supplementation. The patient developed chest pain and an elevated lactate level of 2.1 mmol/L; therefore, the decision to transfuse was made. All crossmatched available units with varying Weiner haplotypes (R0R0, R1--, R2--, rr) were 4+ incompatible. Based on the patient's preliminary phenotype reported by ARC, the decision was made to transfuse a rr, K and Fy(a) negative, split pRBC unit. The split unit was leukoreduced, irradiated, and to be transfused over 3 hours. In preparation for transfusion, the patient was premedicated with 500 mg methylprednisolone and 1 g/kg IVIG. After approximately 55 mL of pRBC was transfused, the patient developed chills and the transfusion was immediately stopped. One hour after the transfusion was stopped, the patient developed a fever with a peak temperature of 39.2°C. Pre- and posttransfusion laboratory results are shown in Table 2.

Pre-transfusion sample MMA results from the ARC were reported posttransfusion and suggested accelerated clearance of antigen positive red blood cells (37.5% reactive monocytes without complement with R1R1 cells, 46.0% without fresh complement with rr cells). *RHD/RHCE* sequencing results from the ARC revealed loss of RHCE antigens including the high prevalence antigen Rh17 and the gain of low prevalence antigen Rh37, consistent with D·· phenotype. A posttransfusion sample was sent to the ARC for adsorption and demonstrated new anti-C and anti-e antibodies.

Day 1 posttransfusion, and 3 days after the eIND process was initiated, the patient began receiving HBOC-201 and ultimately received 17 units over 10 days. An initial loading dose of two units was followed by 1 unit every 12 h as recommended by the manufacturer, with the goal of resolution of symptoms and Hgb > 7 g/dL. The patient began to improve with resolution of chest pain and normalization of lactic acid. Laboratory samples were drawn once daily in the AM. Hemoglobin increased from 2.5 to 7.3 g/dL as measured by standard hematology analyzer assays (XN-10, Sysmex, Kobe Japan). The course was complicated by methemoglobinemia measured via co-oximetry (GEM Premier 5000, Werfen, Barcelona Spain), and the patient was treated with vitamin C without a pause or delay in HBOC-201 administration. Laboratory measurements are shown in Figure 1. The patient

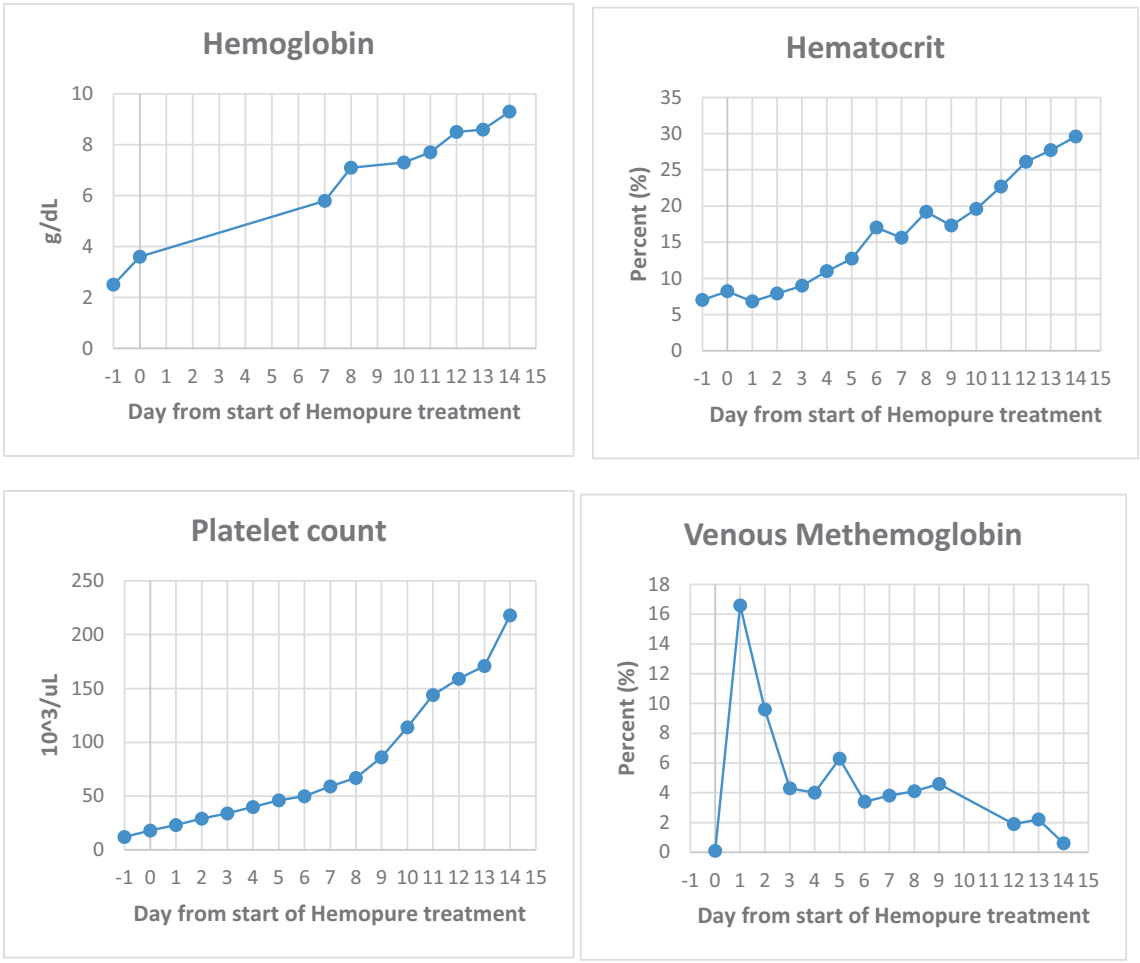


**FIGURE 1** Hematology measures while on HBOC-201 course. [Color figure can be viewed at wileyonlinelibrary.com]

received no further transfusion of any blood products that admission and was discharged in stable condition with a Hgb of 9.7 g/dL. At discharge the patient's functional status had improved to her baseline prior to the initiation of ALL-directed therapy.

## 3 | DISCUSSION

The Rh genotyping results received from the ARC post-transfusion suggested the patient was D·· phenotype which is consistent with the patient's known history of anti-Rh17 antibodies. This phenotype is similar to the D-- phenotype with its complete lack of C/c and E/e antigens including Rh17.[2] However, there is a gain of the Rh37 antigen which has a low frequency of <0.01%.[15] Compatible Rh17 negative units could not be obtained despite extensive national search for donors. Although there is minimal myelosuppression from the utilized chemotherapeutic regimen, future pRBC transfusion is a possibility. Per the ARDP, there are seven compatible Group O, D+ C- c- E- e- donors in the United States with a larger international pool concentrated in Japan.

The decision to transfuse incompatible blood to the patient was not made lightly. Given the patients known antibodies of unknown clinical significance, the risks of transfusion remained extreme. However, in the setting of the patient's life-threatening critical anemia, increasing lactate and new onset symptoms it was determined that the benefits outweighed the risks of transfusion. At the time of transfusion, no clear timeline or approval was known for the Hemopure approval. Pre transfusion medication of 500 mg methylprednisolone and 1 g/kg IVIG was based on practices of prevention of immune mediated hemolysis in hyperhemolysis patients.[16,17] Additional transfusion requirements included one to one monitoring in the ICU, continuous vital sign monitoring and slowest infusion rate of one half unit over 3 h.

The patient experienced chills and fever after the transfusion of incompatible pRBC. Given the patient's decrease in posttransfusion hemoglobin and mild increase in bilirubin, it is possible that the patient

hemolyzed some of the incompatible Rh17 positive RBCs transfused. However, the normal haptoglobin, LDH and negative posttransfusion DAT make hemolysis less likely. The patient experienced previous febrile episodes with a maximal temperature of 38.8°C 2 days prior to transfusion. The patient did not meet National Healthcare Safety Network (NHSN) Hemovigilance Surveillance Module v2.8 criteria for a hemolytic or febrile nonhemolytic transfusion reaction and was deemed to be due to underlying disease.[18] We suspect that had the patient received a larger volume of incompatible blood, evidence of hemolysis would be more prominent, including decreased haptoglobin. The patient developed new anti-e and anti-C antibodies following transfusion of a rr (c,e positive) unit. The new posttransfusion anti-e antibody can be explained by the e positivity of the rr unit. However, the new anti-C antibody cannot be explained. Phenotypes for the units received at an outside hospital could not be confirmed.

During the patient's 10-day course of HBOC-201, the patient experienced transient methemoglobinemia, consistent with known symptoms of HBOC's.[11,19,20] However, Hemopure has been shown to interference with light absorbance at 340, 415, and 520–580 nm while methemoglobin was measured around 555 nm.[21] Thus, while we believe this to be true methemoglobinemia, some interference with co-oximetry measurement due to Hemopure cannot be completely ruled out.

Additionally, during the patient's HBOC-201 course, multiple hemoglobin measurements from Days 2 to 8 could not be performed due to interference flags from the hematology analyzer. The Sysmex hematology analyzer (XN-10) measures hemoglobin using a cyanide-free sodium lauryl sulphate (SLS)-based photometric method. Interference was likely due to the photometric interference from of cell free Hemopure in the plasma. This is consistent with literature that has shown hematology laboratories were not able to consistently measure total hemoglobin as well as mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in patients being treated with HBOC-201.[22] Given the interference of cell free hemoglobin in hematology measurements, a manually spun hematocrit may be followed as the most accurate indicator due to the lack of interference from cell free hemoglobin products.

After the initiation of Hemopure, the patient required no further transfusions of any blood products due to increasing platelet counts and hematocrit (Figure 1). We suspect this to be due to bone marrow recovery after the treatment of the patient's underlying leukemia. Overall, this patient's clinical improvement and recovery seems to support the use of HBOC-201 in patients with life-threatening anemia who cannot receive conventional blood products.

## 4 | CONCLUSION

A patient with life-threatening anemia in the setting of a rare anti-Rh17 antibody was transfused incompatible blood and further treated with HBOC-201. The search for Rh17 negative units is still ongoing and the possibility of repeat transfusion with incompatible pRBC and HBOC-201 remains. This case illustrates the challenge of finding compatible units in a patient with anti-Rh17 antibodies, the dilemma of transfusing incompatible units in a patient with severe anemia and obtaining alternatives to blood products.

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## CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

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## REFERENCES

1. Vege S, Peyrard T, Wagner FF. The Rh system. The technical manual. 21st ed. Maryland: AABB; 2023. p. 337–66.
2. Huang CH, Chen Y, Reid M, Ghosh S. Genetic recombination at the human RH locus: a family study of the red-cell Evans phenotype reveals a transfer of exons 2-6 from the RHD to the RHCE gene. Am J Hum Genet. 1996;59(4):825–33.
3. Reid ME, Lomas-Francis C, Olsson ML. Rh blood group system. The blood group antigen facts book. Third ed. California: Academic Press; 2012. p. 147–262.
4. Sarihi R, Ahmadnejad M, Mohammadi S, Eshghi P, Herfat F, Jolharnejad S, et al. Hemolytic disease of the fetus and newborn caused by anti-Hr0 in a 27-year-old female with Dc- phenotype: a case report. Transfus Apher Sci. 2021;60(1):102913. https://doi.org/10.1016/j.transci.2020.102913
5. Daniels G. Rh and RHAG blood group systems. Human blood groups. 2nd ed. Oxford: Blackwell Science; 2005. p. 182–258.
6. Dajak S, Ipavec N, Cuk M, Golubic Cepulic B, Mratinovic-Mikulandra J, Milardovic J, et al. The outcome of hemolytic disease of the fetus and newborn caused by anti-Rh17 antibody: analysis of three cases and review of the literature. Transfus Med Hemotherapy. 2020;47(3):264–71. https://doi.org/10.1159/000503012
7. Hirose M, Nakanishi K, Kaku S, Moro H, Hodohara K, Aotani H, et al. Fetal hemolytic disease due to anti-Rh17 alloimmunization. Fetal Diagn Ther. 2004;19(2):182–6. https://doi.org/10.1159/000075147

8. Zumberg M, Gorlin J, Griffiths EA, Schwartz G, Fletcher BS, Walsh K, et al. A case study of 10 patients administered HBOC-201 in high doses over a prolonged period: outcomes during severe anemia when transfusion is not an option. Transfusion. 2020;60(5):932–9. https://doi.org/10.1111/trf.15778
9. Expanded Access Protocol for the Treatment Use of HBOC-201. 2021. Accessed December 1, 2023. https://clinicaltrials.gov/study/NCT01881503
10. Sen GA. Hemoglobin-based oxygen carriers: current state-of-the-art and novel molecules. Shock Augusta Ga. 2019;52(1S Suppl 1):70–83. https://doi.org/10.1097/SHK.0000000000001009
11. Mackenzie CF, Dubé GP, Pitman A, Zafirelis M. Users guide to pitfalls and lessons learned about HBOC-201 during clinical trials, expanded access, and clinical use in 1,701 patients. Shock Augusta Ga. 2019;52(1S Suppl 1):92–9. https://doi.org/10.1097/SHK.0000000000001038
12. Jahr JS, Mackenzie C, Pearce LB, Pitman A, Greenburg AG. HBOC-201 as an alternative to blood transfusion: efficacy and safety evaluation in a multicenter phase III trial in elective orthopedic surgery. J Trauma. 2008;64(6):1484–97. https://doi.org/10.1097/TA.0b013e318173a93f
13. Katz LM, Manning JE, McCurdy S, Sproule C, McGwin G Jr, Moon-Massat P, et al. Nitroglycerin attenuates vasoconstriction of HBOC-201 during hemorrhagic shock resuscitation. Resuscitation. 2010;81(4):481–7. https://doi.org/10.1016/j.resuscitation.2009.12.015
14. Natanson C, Kern SJ, Lurie P, Banks SM, Wolfe SM. Cell-free hemoglobin-based blood substitutes and risk of myocardial infarction and death: a meta-analysis. JAMA. 2008;299(19):2304–12. https://doi.org/10.1001/jama.299.19.jrv80007
15. Cheng GJ, Chen Y, Reid ME, Huang CH. Evans antigen: a new hybrid structure occurring on background of D·· and D-- Rh complexes. Vox Sang. 2000;78(1):44–51. https://doi.org/10.1159/000031148
16. Aragona E, Kelly MJ. Hyperhemolysis in sickle cell disease. J Pediatr Hematol Oncol. 2014;36(1):e54–6. https://doi.org/10.1097/MPH.0b013e31828e529f
17. Rihsling A, Simeunovic H, Sanchez S, Henny C, Lejon Crottet S, Mansouri Teleghani B, et al. "Don't add fuel to the fire"–hyperhemolysis syndrome in a pregnant woman with compound sickle cell disease/β0-thalassemia: case report and review of the literature. Acta Haematol. 2023;146(5):1–10. https://doi.org/10.1159/000533776
18. National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol v2.8. 2023. https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf
19. Bari S, Rabinovich M, Curry M, Jain SR, Cafuir L. Use of hemoglobin based oxygen carrier HBOC-021 (Hemopure) As a bridge during emergencies in patients unable to receive blood products: experience at a tertiary care center. Blood. 2017;130:4926. https://doi.org/10.1182/blood.V130.Suppl_1.4926.4926
20. Sherbeck JP, Cooling L, Davenport RD, Yamada C. Significant methemoglobinemia with bovine hemoglobin infusion in a case with severe autoimmune hemolytic anemia. Transfusion. 2016;56(3):777–8. https://doi.org/10.1111/trf.13441
21. Vera MA, Eid T, El-Khoury JM. Effects of bovine hemoglobin on chemistry testing: Pseudohemolysis or real? Clin Chem. 2022;68(4):607–8. https://doi.org/10.1093/clinchem/hvab222
22. Bronkhorst-van der Helm MW, Weerkamp F, Huisman A, Huisman EJ, Russcher H. Interference of bovine hemoglobin-based oxygen carrier-201 (Hemopure) on four hematology analyzers. Int J Lab Hematol. 2023;45(6):869–74. https://doi.org/10.1111/ijlh.14146

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