NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

# Battle of the Blood:

## Hemolytic Disease of the Fetus and Newborn

*by*
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## Preparation

Before beginning this case study, please review the following information about the ABO and Rh blood classifications systems and the life cycle of an erythrocyte:

- Interactive Biology. (2022). Blood types physiology (ABO and Rh blood groups). [Video]. Running time: 5:52 min. YouTube. <https://youtu.be/99uGL0lJ0Es>
- Cleveland Clinic. (2022). Rh factor. [Webpage]. Cleveland Clinic. <https://my.clevelandclinic.org/health/diseases/21053-rh-factor>
- Hasudungan, A. (2014). Haematology: Red blood cell life cycle. [Video]. Running time: 6:13 min. YouTube. <https://youtu.be/cATQFej6oAc>

## Part I –ABO and Rh Classification Systems

Elijah and Annie had been excited to learn of Annie's second pregnancy. It was now several weeks later, and they were about to walk into their 20-week ultrasound appointment to find out the gender of their baby. During Annie's first pregnancy, this was something they greatly enjoyed. In addition to finding out the gender, Elijah and Annie had decided to have an amniocentesis performed, like they did in their first pregnancy, to check for any genetic or chromosomal abnormalities. Their first daughter had Down syndrome, and they wanted to be prepared in case their second child had it as well.

Dr. White, their OB/GYN, entered the room. "Good to see you again, Annie. How are you feeling this afternoon?"

"I'm doing well. This pregnancy has been a lot easier than my last one. We're very excited to find out the gender of our baby, but we're worried about the results of our amniocentesis."

"I understand that waiting for the test results can be stressful," replied the doctor. "I assure you that no matter what the results are, we'll go over them in detail and discuss what they mean for your baby. Do you have any other questions or concerns for me?"

"Yes. I remember that during my last pregnancy I had to receive a shot at some point in my third trimester, but I don't recall why. Can you explain what that shot was for?"

Dr. White began, "The shot that you were given is called RhoGAM treatment..."

The ABO and Rh classifications are two of numerous blood classification systems. Both systems classify an individual's blood type based on the presence (or absence) of erythrocyte cell-surface markers. These cell surface markers are called antigens (e.g., antigen A, antigen B, and antigen Rh). Antigens A, B, and Rh are genetically determined.

In addition to differences in erythrocyte antigen composition, the type of antibodies varies between blood types. Generally speaking, an individual develops antibodies after exposure to a non-self-antigen. If an individual is exposed to antigen not associated with their own body, the individual's immune system responds by making antibodies. Antibodies enhance the immune system's clearance of non-self-antigens. Antibodies are proteins that bind to complementary antigens, forming an antibody-antigen complex (e.g., anti-A antibodies bind to A antigens). The binding of the antibody to the antigen serves to neutralize the antigen or to alert the infection fighting immune cells. The development of antibodies following antigen exposure is referred to as *sensitization*. A subsequent exposure to the antigen results in amplified immune response, i.e., a rapid and exponential increase in antibody production.

Although the reason is not completely understood, anti-A and anti-B antibodies are present at birth and require no sensitization. The development of anti-Rh antibodies requires sensitization. (Anti-Rh antibodies are also referred to as anti-D antibodies.) To develop anti-Rh (anti-D) antibodies, an Rh– individual must be exposed to an Rh antigen (also known as a D antigen). The same is not true for anti-A and anti-B antibodies. Therefore, anti-A and anti-B antibodies are referred to as *isohemagglutinin* or naturally occurring antibodies whereas anti-Rh antibodies are referred to *alloantibodies* (antibodies requiring a sensitization event).

## Questions

*Important note:* In answering all questions in this case study, unless otherwise noted, assume that sensitization has already occurred.

1. What is sensitization?

2. Complete Table 1 to summarize your understanding of the ABO blood typing system. For each blood type, circle the antigens present on the erythrocyte and the antibodies present in the plasma.

   *Table 1*. Characteristics of different blood types.

| *Blood Type* | *Antigens (present on erythrocyte)* | *Antibodies (present in plasma)* |
|---|---|---|
| A+ | A B Rh | anti-A anti-B anti-Rh |
| A– | A B Rh | anti-A anti-B anti-Rh |
| B+ | A B Rh | anti-A anti-B anti-Rh |
| B– | A B Rh | anti-A anti-B anti-Rh |
| AB+ | A B Rh | anti-A anti-B anti-Rh |
| AB– | A B Rh | anti-A anti-B anti-Rh |
| O+ | A B Rh | anti-A anti-B anti-Rh |
| O– | A B Rh | anti-A anti-B anti-Rh |

3. Demonstrate your understanding of the ABO and Rh blood typing classification systems by adding the appropriate antigens and antibodies to the erythrocyte and plasma, respectively, in Figure 1 below.

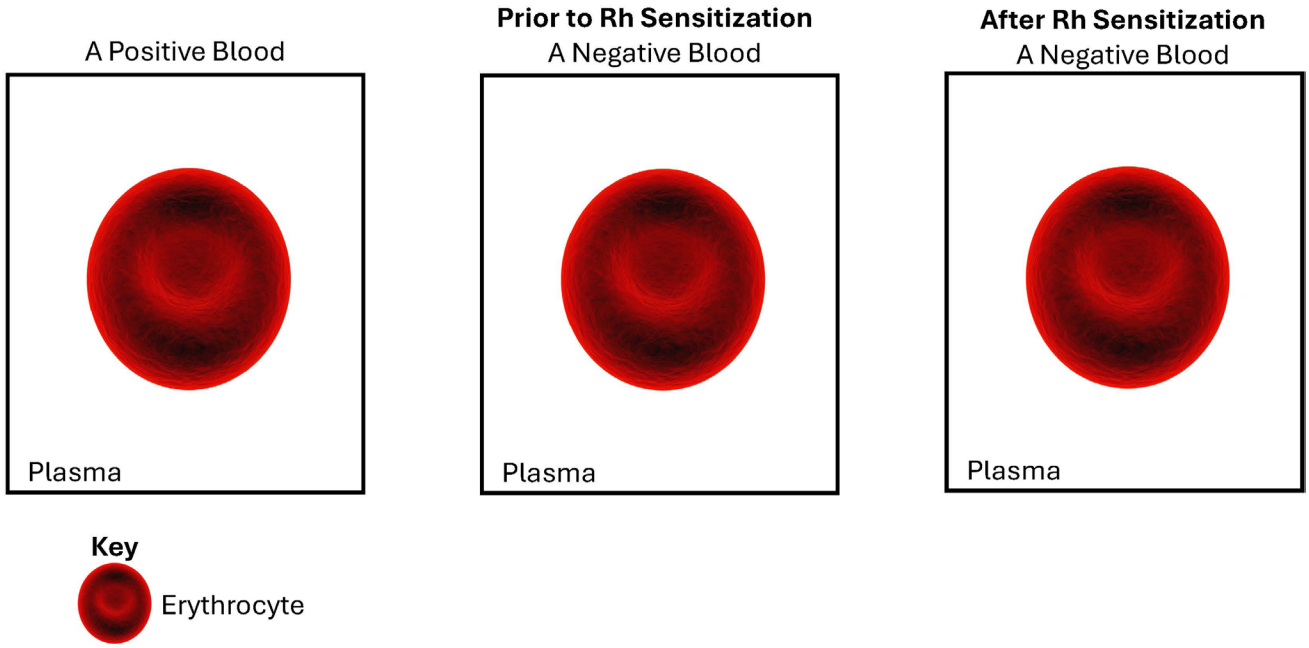


*Figure 1*. A pictorial representation of A+ and A– blood.

4. What is agglutination and how does it relate to blood transfusions? Please use the terms *antibodies* and *antigens* in your response.

5. Blood transfusions may be given as whole blood transfusions or erythrocyte only transfusions. In other cases, individuals may receive plasma only transfusions. Complete Table 2 to describe the substances present in each type of transfusion.

*Table 2*. Composition of transfusions.

| *Transfusion Type* | *Erythrocyte Antigens (e.g., antigen A, antigen B, antigen Rh)* | *Antibodies (e.g., anti-A antibodies, anti-B antibodies, anti-Rh antibodies)* |
|---|---|---|
| Erythrocyte only | Yes or No | Yes or No |
| Plasma only | Yes or No | Yes or No |
| Whole blood transfusion | Yes or No | Yes or No |

6. Apply your knowledge from the previous question to determine the antigen(s) and antibody/antibodies present in the donor's whole blood and or erythrocyte-only transfusions for the listed blood types. In Table 3 below, circle the substances that are present.

*Table 3*. Composition of whole blood vs. erythrocyte only transfusions.

| *Transfusion Type* | *Whole Blood* | *Erythrocyte Only* |
|---|---|---|
| AB+ | A antigen<br>B antigen<br>Rh antigen<br>anti-A antibodies<br>anti-B antibodies<br>anti-Rh antibodies | A antigen<br>B antigen<br>Rh antigen<br>anti-A antibodies<br>anti-B antibodies<br>anti-Rh antibodies |
| AB– | A antigen<br>B antigen<br>Rh antigen<br>anti-A antibodies<br>anti-B antibodies<br>anti-Rh antibodies | A antigen<br>B antigen<br>Rh antigen<br>anti-A antibodies<br>anti-B antibodies<br>anti-Rh antibodies |
| O+ | A antigen<br>B antigen<br>Rh antigen<br>anti-A antibodies<br>anti-B antibodies<br>anti-Rh antibodies | A antigen<br>B antigen<br>Rh antigen<br>anti-A antibodies<br>anti-B antibodies<br>anti-Rh antibodies |
| O– | A antigen<br>B antigen<br>Rh antigen<br>anti-A antibodies<br>anti-B antibodies<br>anti-Rh antibodies | A antigen<br>B antigen<br>Rh antigen<br>anti-A antibodies<br>anti-B antibodies<br>anti-Rh antibodies |

7. An individual receiving a blood transfusion may receive a whole blood transfusion (red blood cells, platelets, and plasma) or a red blood cell only transfusion. An individual with O+ blood can receive an O+ or O– red blood cell transfusion but can only receive a whole blood donation from someone with O+ (not O–) blood. Explain this dichotomy.

8. Type ____ blood is considered the universal donor. All ABO+ and ABO– blood types can safely receive an erythrocyte only transfusion of this type of blood. Circle the correct answer.

A+ A– B+ B– AB+ AB– O+ O–

9. Type ____ blood is considered the universal recipient. This type of blood does not contain antibodies against A, B or Rh antigens. Therefore, this type of blood can receive erythrocyte donations from any of the ABO+ or ABO– blood types. Circle the correct answer.

A+ A– B+ B– AB+ AB– O+ O–

10. The doctor indicated the reason Annie needed a shot (RhoGAM) in her third trimester was due to her blood type. Annie's blood type is A–. Visit the website listed below to learn about RhoGAM and then explain why it is essential for an OB/GYN doctor to know a pregnant mother's blood type.
    - Kedrion Biopharma Inc. (2025). Your baby's life story begins: the life-saving science behind RhoGAM. [Webpage]. <https://www.rhogam.com/rhogam-science/>

## Part II – Chart Review

After Dr. White finished her explanation of RhoGAM treatment, she smiled at Annie and said, "I know that's a lot of information for you to take in. But the reason I went over it is because as I was reviewing your chart, I was reminded that your blood type is A–. When we performed an amniocentesis on your first child, we found out that her blood type was incompatible with yours. Because of this, we administered a RhoGAM shot at your first pregnancy's 28-week appointment and again after you gave birth to prevent you from developing anti-Rh antibodies."

### *Question*

11. Prior to RhoGAM availability, mothers with Rh– blood would become sensitized to the Rh antigen during the delivery of an Rh+ baby. RhoGAM is now able to prevent this. Complete Table 4 by answering "yes" or "no" to determine whether the antibody type is present in each circumstance for a biological female with A– blood. Assume for the pregnancy the child had Rh+ blood and the mother and child's blood mixed at birth.

*Table 4.* Antibodies present in biological female with A– blood.

| | *Antibodies Present* | | |
|---|---|---|---|
| *Circumstance* | *Anti-A* | *Anti-B* | *Anti-D* |
| Prior to pregnancy/sensitization | | | |
| After first pregnancy, assuming successful RhoGAM treatment | | | |
| After first Pregnancy, assuming delayed and unsuccessful RhoGAM treatment | | | |

## Part III – Coombs Testing

"Annie," continued Dr. White, "as I stated previously, we administered RhoGAM during your last pregnancy with the goal of preventing you from developing anti-Rh antibodies. However, just to be safe, we will need to do a test to ensure that the RhoGAM was successful. We are going to use an indirect Coombs test to figure that out. The test results will let us know if your blood contains anti-Rh antibodies."

A direct Coombs test detects antibodies that are already present on and attached to red blood cells. An indirect Coombs test detects the presence of antibodies in the plasma. Please familiarize yourself with the indirect Coombs test by watching the following video:

- Medicosis Perfectionalis. (2018). Indirect Coombs test [Video]. Running time: 2:15 min. YouTube. <https://youtu.be/czHuMY36Jss>

### *Questions*

12. Typically, a direct Coombs test is ordered for newborns after birth and aids in diagnosing hemolytic anemia. If there are concerns during pregnancy about Rh incompatibility, the mother may get an indirect Coombs test. Explain why the direct Coombs test would provide diagnostic information in the newborn, but not in the mother.

13. Interpret the results of the indirect Coombs test shown in Figure 2. Write a paragraph in which you describe the results of this test. In your response, be sure to address the following:
    a. whether the results of this test most likely associated with an individual who did or did not receive RhoGAM treatment (explain why);
    b. the goal of RhoGAM treatment;
    c. the results of the patient's indirect Coombs test (positive or negative); and
    d. whether or not the patient's plasma contains anti-Rh antibodies.

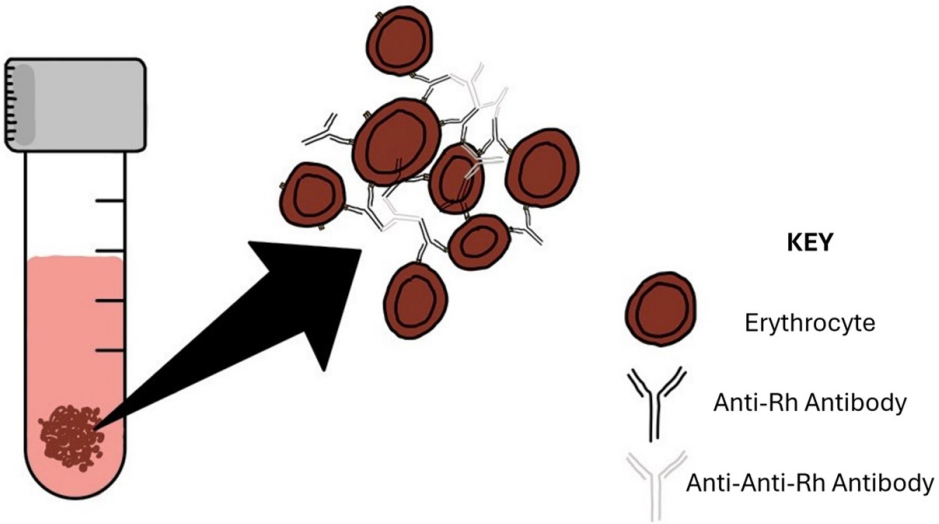


*Figure 2*. Results of the indirect Coombs test. *Credit:* Jeremiah J. Renfrow.

## Part IV – Preventive Care

A few days after her office appointment, Dr. White called Annie. "Good news," said the doctor. "The results of your indirect Coombs test came back negative; your plasma did not contain anti-Rh antibodies in your plasma. This means that the RhoGAM treatment we used during your first pregnancy was successful."

Annie's access to adequate and early prenatal care in her first pregnancy prevented her from being sensitized to the Rh(D)-antigen and developing anti-Rh(D) antibodies. Unfortunately, not all pregnant women have access to adequate prenatal care, an important variable in improving health outcomes for the mother and child.

The American College of OB/GYNs recommends administering the RhoGAM shot during the 28$^{th}$ week of pregnancy and within 72 hours of childbirth; expectant mothers receiving both doses of RhoGAM only have a 0.1 to 0.2% chance of becoming sensitized. Unfortunately, in the United States, as of 2023 only 75.2% of expecting mother's received adequate prenatal care (Office of Disease Prevention and Health Promotion, 2025). Failure to receive RhoGAM treatment at the appropriate time is one form of inadequate care. Receiving the RhoGam shot at a later date reduces its effectiveness to 88%.

Prior to RhoGAM treatment, an Rh– mother was typically able to give birth to her first Rh+ baby without complications. However, following the birth of a Rh+ baby, it was not uncommon for the Rh– mother to have repeated pregnancies that ended in miscarriage or resulted in the birth of a "blue baby." These babies were so named due to the bluish appearance to their skin and usually died shortly after birth. These blue babies experienced a condition now referred to as hemolytic disease of the newborn and fetus (HDFN). (*Hemolytic* is a medical term used to describe premature destruction of erythrocytes.) Appropriate prenatal care can prevent such a tragedy.

### *Questions*

14. Explain why, prior to RhoGAM treatment, Rh– women who were pregnant with their second (or subsequent) Rh+ fetus often gave birth to "blue babies." (*Hint:* red blood cells do not cross the placenta, but antibodies do.)

## Part V – Consequences of HDNFN

This case is fictional, but also realistic. Annie and her baby were fortunate, but things do not always turn out so well. Consider the case of baby Mack, a real patient with hemolytic disease of the newborn and fetus (HDFN).

HDNF is a disease in which the maternal anti-Rh(D) antibodies cross the placenta, bind to and destroy the fetal erythrocytes, leading to additional strain the fetal heart, liver, and kidney. The heart is responsible for delivering oxygen-rich erythrocytes to the body tissues; the liver and kidneys assist in the process of erythrocyte recycling.

Treatment of HDNF can include blood transfusions or phototherapy (the use of light to speed the catabolism of metabolites associated with increased erythrocyte destruction). Baby Mack received 14 intrauterine (prior to birth) blood transfusions and 14 blood transfusions post birth. His mother Tiffany did not receive RhoGAM in her first Rh-incompatible pregnancy. Prenatal healthcare plays a vital role in supporting the health of the mother and infant, including preventing and treating HDFN. You can read more about baby Mack at:

- Community Blood Center of Ozark's. (2025). Rh disease leads to 28 transfusions for baby Mack. [Webpage]. <https://www.cbco.org/rh-disease-leads-to-28-transfusions-for-baby-mack/>

Answer the questions below to review the process of erythrocyte recycling and to explore the pathophysiology of HDNF.

### *Questions*

15. Fill in the blanks using the provided word bank to complete the steps of RBC recycling carried out by the spleen, liver, and bone marrow. Not all terms must be used.

| *Word Bank* | | |
|---|---|---|
| bile | feces | amino acids |
| iron | globin | heme |
| oxygen | spleen | erythropoiesis |
| 120 | bone marrow | urine |
| hemoglobin | bilirubin | liver |
| | macrophages | |

a. Erythrocytes are responsible for carrying _____ throughout the bloodstream and typically have a lifespan of _____ days.

b. Blood cell removal occurs in three organs, including the _____, _____, and _____ _____.

c. In the spleen, cells called _____ engulf old and worn-out erythrocytes, breaking them down.

d. This process results in excessive amounts of _____, which is further broken down into _____ and _____.

e. Globin is converted into _____ _____; heme is further reduced into its component parts of _____ and _____.

f. The bilirubin is transported to the liver, where it is processed to become water-soluble and is then secreted with the _____, ultimately disposed of through the _____ and _____.

g. Nutrients are reabsorbed in the small intestine to be reused for the process of _____.

16. Old and worn-out red blood cells are broken down and recycled primarily to recover an essential resource for the body. What is the main purpose of this process? Choose the best answer from the following options:
    a. To increase the number of white blood cells available to fight infections.
    b. To reclaim iron needed to produce new red blood cells, which cannot be supplied quickly enough through diet alone.
    c. To regulate blood pressure by reducing the total volume of circulating blood.
    d. To filter and remove harmful toxins from the blood more effectively.

17. Table 5 lists some of the conditions associated with HDFN. In the column labeled "cause," describe how or why the increased destruction of erythrocytes would lead to each of these conditions.

*Table 5*. HDFN symptoms and causes.

| *Condition* | *Description* | *Cause* |
|---|---|---|
| Anemia | Insufficient number of red blood cells or hemoglobin. | |
| Hypoxia | Insufficient oxygen in the body tissues to sustain bodily functions. | |
| Hyperbilirubinemia (jaundice) | Yellow discoloration of the body tissue resulting from the accumulation of excess bilirubin. | |
| Fetal heart strain | Increased workload of the fetal heart. | |

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