COURSE DESCRIPTION

Transfusion of blood and blood products can be a life-saving medical procedure. This continuing education course will identify the importance of the ABO and Rh blood group systems, general information on blood donation, the types of blood components, laboratory testing for compatibility, transfusion reactions, and hemolytic disease of the newborn.

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OBJECTIVES

Upon completion of the continuing education course, the healthcare professional will be able to:

1. Define antigen and antibody.
2. Identify the antigens and antibodies in the ABO system.
3. Determine an ABO type given the results of forward and reverse typing.
4. State the importance of the naturally occurring antibodies in the ABO system as related to blood transfusion.
5. Describe the importance of the D antigen in the Rh blood group system as it relates to blood transfusion and Rh hemolytic disease of the newborn.
6. Describe basic laboratory tests performed in the hospital blood bank.
7. Describe blood donation, including screening procedures to protect against transmission of certain diseases or potential harm to the individual.
8. Compare single donor and apheresis collections.
9. Identify specimen collection requirements for blood bank laboratory testing.
10. Describe the importance of patient identification and the results of clerical errors.
11. Describe the fractionation of whole blood into components and plasma derivatives.
12. Identify indications for transfusion of blood components and plasma derivatives.
15. Name other blood group systems that can cause hemolytic disease of the newborn.
16. Identify agencies involved in the regulation and accreditation of donor centers and transfusion services

Disclaimer

The writers for NCCT continuing education courses attempt to provide factual information based on literature review and current professional practice. However, NCCT does not guarantee that the information contained in the continuing education courses is free from all errors and omissions.
INTRODUCTION

Transfusion of blood and blood products can be a life-saving medical procedure. The following statistics are from the American Red Cross.

- Every two seconds someone in the United States needs blood.
- Approximately 36,000 units of red blood cells are needed every day in the United States.
- Nearly 7,000 units of platelets and 10,000 units of plasma are needed daily in the United States.
- Nearly 21 million blood components are transfused each year in the United States.

Before a transfusion can occur, donor blood must be collected, tested, and fractionated into components; and the recipient of the blood transfusion must have his/her blood tested for compatibility with the blood components selected for transfusion.

The donor and recipient laboratory testing is detailed, and accuracy is of the utmost importance. Transfusion of an incompatible blood type can result in death of the recipient.

This continuing education course will identify the importance of the ABO and Rh blood group systems, general information on blood donation, the types of blood components, laboratory testing for compatibility, transfusion reactions, and hemolytic disease of the newborn.

WHAT IS A BLOOD BANK?

Historically the term ‘blood bank’ has been used for three entirely different areas:

1. the location where blood was donated,
2. the location where laboratory testing is performed before a transfusion, and
3. the refrigerator used to store blood.

Technically the only true correct use of ‘blood bank’ is #3. A ‘blood bank’ is a type of refrigerator that stores units of red blood cells and other blood components at temperatures of 1°-6°C.

TRANSFUSION SERVICES / IMMUNOHematology

In a hospital setting, the department of the laboratory that performs testing related to blood transfusion is more appropriately called ‘Transfusion Services’ or ‘Immunohematology’. However, in many locations it will always affectionately be known as the ‘Blood Bank’.

Clinical laboratory scientists in Transfusion Services perform testing to assure safe transfusion of blood and blood products to patients. This includes blood typing, pretransfusion testing, compatibility testing, and selection of appropriate blood products for transfusion for a patient. Additional tasks performed in Transfusion Services include
receiving and retesting blood and blood components received from donor centers, preparing blood components for transfusion, releasing blood and blood components to healthcare providers for transfusion to the recipient, accounting for the disposition of blood or components, and follow up testing when a transfusion reaction is suspected.

BLOOD CENTERS / DONOR SERVICES

Blood centers specialize in donor services. These facilities collect, process, fractionate, test, and store blood and blood components for distribution to hospitals for transfusion to people. Blood centers are managed by the American Red Cross, communities, and the Armed Services.

Most blood centers also provide reference laboratory testing for identification of unexpected red blood cell antibodies, and obtaining blood for people with very rare blood types. The reference laboratory also provides testing for abnormal platelet destruction problems. Donor center physicians are also available for consultation with patient physicians regarding transfusion with blood and blood components.

A BRIEF HISTORY OF TRANSFUSION MEDICINE

Even in ancient times, it was known that loss of blood frequently resulted in weakness and death. The first attempts at blood transfusion began after British physician William Harvey described the circulation and properties of blood in 1628. The first successful transfusion was in 1665 and it was between two dogs – one was bled almost to death and then transfused with blood from another. Both dogs survived. In 1667, about 8 ounces of blood from a sheep was transfused to a 16-year-old boy who survived. Other transfusions from cows to man were performed around this time, without beneficial effects, and often resulted in death. Most of these attempts were in England and France, and both countries eventually placed a ban on animal to human transfusions.

The date of the first documented transfusion of human blood is December 22, 1818. In England, a 35 year-old-man with what is now considered to be gastric carcinoma was transfused with about 14 ounces of blood from various human donors. The blood was administered in small amounts via a syringe at intervals of 5-6 minutes. The patient’s symptoms temporarily improved but he died within a week, most likely due to his disease. The procedure was performed by James Blundell, a well-known physician, physiologist, and obstetrician of his day. His interest in blood transfusion stemmed from seeing women die from hemorrhage following childbirth. Dr. Blundell and one of his colleagues, Henry Doubleday, practiced transfusion in cases of post-partum hemorrhage with various stages of success for many years.

The discovery of the ABO blood group system marked the beginning of modern blood banking and transfusion medicine. Karl Landsteiner was the scientist who was instrumental in this landmark discovery in the year 1901. He identified the presence of the A and B antigens on red blood cells and the corresponding antibodies in serum. The ABO blood group still represents one of the primary tests performed in pre-transfusion testing and remains the single most influential factor in transfusion reactions with negative outcomes.
The second major historical event that proved instrumental in transfusion of blood and blood components was discovery of the Rh blood group system. The major component of the Rh blood group system is the D antigen. The anti-D antibody was identified in 1939 by Philip Levine and Rufus Stetson. In 1940, Karl Landsteiner and Alexander Weiner discovered the corresponding D antigen.

The ABO and Rh blood group systems are the most important systems in transfusion medicine. However, many other blood group systems have been identified. Approximately 300 unique antigens have been identified on the surface of red blood cells. These additional antigens and their corresponding antibodies may play a role in transfusion medicine.

ANTIGENS AND ANTIBODIES

To understand blood group systems and transfusion medicine, a working knowledge of antigens and antibodies is needed. Antigens and antibodies are part of the immune system and it is the presence of antigens on the surface of red blood cells that makes blood group systems so vital for transfusion.

Antigens are proteins and polysaccharides found on the surface of cells, and it is the presence of these antigens that make the cells unique. The arrangements of the molecular substances in antigens make them exquisitely precise so that no one antigen is like another one. For example, viruses, pollen, bee venom, and bacteria have antigens on the surface of their cells. The antigens on the chicken pox virus are different from the antigens on the influenza virus just as the antigens on the *Staphylococcus* bacteria are different from the antigens on the *Salmonella* bacteria.

Human antigens are found on red blood cells, white blood cells, platelets, tissue cells, cells that make up organs, as well as in substances found in plasma and other body fluids. Each individual's cells have antigens that are determined genetically; i.e., inherited from the mother and father. By definition, an antigen is any substance that stimulates the immune system to produce an antibody. The human body is designed not to recognize 'self'; i.e., his/her own antigens. Therefore, in normal circumstances, antibodies are not made against antigens on an individual's own cells.

Antibody production is part of the immune system’s protective mechanism. The antibodies bind with antigens to inactivate them so other parts of the immune system can destroy and remove the foreign substance before it can cause damage. Some antigens are ‘strongly antigenic’ and elicit the production of antibodies very quickly. Others antigens are ‘weakly antigenic’ and may not produce many, if any, antibodies. The antigen’s degree of foreignness, the molecular weight, the chemical composition, and the complexity of its structure all influence the production of antibodies.

Biochemically, antibodies are a type of protein called immunoglobulins. They are found primarily in the plasma, but other body fluids can contain antibodies. The general types of antibodies are IgM, IgG, IgA, IgE, and IgD, with ‘Ig’ being the abbreviation for immunoglobulin. Each type of antibody serves its own purpose in the function of the immune system and each share a common structure as on the following page.
Antibodies in blood group systems are almost exclusively IgM and IgG.

Many laboratory procedures rely on the detection of antigens, antibodies, and their reactions. Examples follow.

- **Determination of the type of lymphoma:** A flow cytometer is used to detect antigens on the surface of lymphocytes. Cells from the lymph nodes and/or bone marrow are added to a solution containing antibodies coupled to fluorescent dyes. The flow cytometer is able to detect antigen:antibody interactions and, following analysis, the antigens on the lymphocytes can be determined, thus identifying the type of lymphoma.

- **Exposure to syphilis:** The Rapid Plasma Reagin (RPR) test is performed to identify the presence of a non-specific antibody that may indicate exposure to the bacteria that causes syphilis. If this test is positive, other tests must be performed to confirm the individual had an exposure to the syphilis bacteria.

- **Diagnosis of hepatitis:** Antigen and antibody tests are performed to identify infection with hepatitis A, hepatitis B, or hepatitis C. These tests include hepatitis A antibody, IgM; hepatitis B core antibody, IgM; hepatitis B surface antigen; and hepatitis C antibody. These tests are often automated enzyme immunoassays.

- **All procedures in Transfusion Services are based on antigen:antibody reactions. Most often these reactions are visualized by hemagglutination (agglutination of red blood cells).** Hemagglutination is visualized in the graphic on the following page.
THE ABO BLOOD GROUP SYSTEM

The ABO blood group system is the most important system in blood transfusion as transfusion of an incompatible ABO blood type can result in a life-threatening transfusion reaction and death. This blood group system also has a unique characteristic not found in any other system.

The presence or absence of the A and B antigen on red blood cells determines the blood type.

<table>
<thead>
<tr>
<th>If.....</th>
<th>Then.....</th>
</tr>
</thead>
<tbody>
<tr>
<td>the A antigen is present</td>
<td>the blood type is group A</td>
</tr>
<tr>
<td>the B antigen is present</td>
<td>the blood type is group B</td>
</tr>
<tr>
<td>the A and B antigens are present</td>
<td>the blood type is group AB</td>
</tr>
<tr>
<td>no A or B antigens are present</td>
<td>the blood type is group O</td>
</tr>
</tbody>
</table>

As with all blood group systems, ABO antigens are inherited. Each individual receives one blood type gene (A, B, or O) from each parent. Each person’s blood type (or “group”) is determined by the set of two genes they inherited (one from each parent).

The set of blood type genes inherited is referred to as the genotype. The genotype determines which blood group is actually displayed, or “expressed.” The blood type expressed by an individual is referred to as their phenotype, which will be A, B, AB, or O.

The A antigen and B antigen are considered “co-dominant” because both the A and B antigens, if one of each has been inherited, will be expressed together. This results in the group AB phenotype.

On the contrary, an individual must inherit an O gene from each parent to express type O blood. The O gene is recessive and two copies are required for that phenotype. The O gene actually contributes no detectable antigen on the surface of red blood cells. A lack of A or B results in type O.

With types A and B, however, an individual only needs to have inherited one copy of either the A antigen or the B antigen gene. For example, if an individual inherits an A gene from one parent and an O gene from the other parent, they will still express type A
blood even though they have one A gene and one O gene (written as AO). This genotype still results in an A phenotype because the A antigen gene is dominant and the O gene contributes no detectable antigen. The same goes for the BO genotype (one B gene and one O gene results in type B blood). Inheriting two A genes also results in type A blood (genotype AA). Likewise, inheriting two B genes results in type B blood (genotype BB).

The following table shows the possible genotypes with each blood type.

<table>
<thead>
<tr>
<th>Blood Type Phenotype</th>
<th>Genotypes that are possible with this blood type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>AA or AO</td>
</tr>
<tr>
<td>B</td>
<td>BB or BO</td>
</tr>
<tr>
<td>AB</td>
<td>AB only</td>
</tr>
<tr>
<td>O</td>
<td>OO only</td>
</tr>
</tbody>
</table>

In the laboratory, a blood type test will not distinguish between an AA or AO, or a BB or BO. A person with a genotype of AO will test as group A, just as a person with a genotype of AA will. Patients are reported as one of the following: A, B, AB, or O.

The following table lists all of the possible genotypes that offspring can inherit based on the blood types and all possible genotype combinations of the parents.

As evidenced by this table, it is possible for ‘Parent 1’ to be group A, ‘Parent 2’ to be group B, and the offspring to be type O. It is not possible for two type O parents to produce offspring other than type O.
The only way to identify an individual’s genotype as AA, AO, BB, or BO is to know the blood type of his/her parents. For example, if an individual knows his blood type is group B and his mother’s blood type is group O, he knows his genotype is BO, as he could have only inherited an O gene from his mother.

The ABO antigens are also found on a variety of tissues, including epithelial and endothelial cells. Some individuals also have soluble forms of the antigens in their fluids such as saliva and urine. In certain diseases and illnesses, individuals can temporarily ‘acquire’ or ‘lose’ their ABO antigens, making it difficult to confirm their blood type and supply blood products for transfusion.

**ABO SYSTEM ANTIBODIES**

The ABO system has a characteristic not found in any of the other blood group systems. This characteristic is the presence of strong naturally-occurring antibodies to the A and B antigens, whichever is lacking on the red cells.

The table below illustrates the antibodies present in the four blood types.

<table>
<thead>
<tr>
<th>Blood type</th>
<th>Antigen(s) on red blood cells</th>
<th>Corresponding naturally occurring antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>A and B</td>
<td>none</td>
</tr>
<tr>
<td>O</td>
<td>none</td>
<td>Anti-A, Anti-B, and Anti-A,B</td>
</tr>
</tbody>
</table>

Anti-A and anti-B are in the IgM class of antibodies. Individuals with blood group O have a third antibody called anti-A,B. It reacts with blood cells from individuals with group A, B, and AB blood types. Anti-A,B is in the IgG class of antibodies.

The presence of these strong naturally-occurring antibodies makes transfusion of compatible ABO blood types of the utmost importance. If an individual receives a transfusion with an ABO type for which he/she has the corresponding antibody, a serious transfusion reaction will occur. For example, if a type A woman with anti-B in her plasma receives a transfusion of type B blood, the woman’s anti-B will attack and destroy the transfused blood with the B antigen. The result of the antigen:antibody interaction is agglutination which results in the red blood cells hemolyzing. Massive amounts of hemoglobin are released into the circulation which can result in a life-threatening situation.
TRANSFUSIONS OF RED BLOOD CELLS

Donated whole blood is fractionated for transfusion purposes. Using centrifugation at the donor centers, donor blood is divided into components: red blood cells, plasma, platelets, and cryoprecipitate.

Although ABO identical red blood cells are desirable for transfusion, red blood cell transfusions do not need to be of the identical blood type. Transfused blood only needs to be a compatible blood type. For example, type O red blood cells are, in theory, compatible with all recipients’ blood types. This is because the type O red blood cells lack the A and B antigens on their surface. This is why type O blood donors are considered ‘universal donors.’

Type O recipients, however, can only receive red blood cell transfusions from other type O donors. This is because a type O recipient has both naturally occurring anti-A and Anti-B antibodies. A type O recipient will react against type A, type B, and type AB red blood cell transfusions.

On the contrary, type AB is considered to be the ‘universal recipient’ as an individual with type AB blood makes neither anti-A nor anti-B antibodies. A type AB recipient can receive blood from type A, type B, type AB, or type O donors.

Type AB blood donors’ red blood cells are only compatible with other AB recipients.

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>Compatible Blood Types for Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (has anti-B)</td>
<td>A, O (contain no B antigen)</td>
</tr>
<tr>
<td>B (has anti-A)</td>
<td>B, O (contain no A antigen)</td>
</tr>
<tr>
<td>AB (no antibodies)</td>
<td>A, B, AB, O (can have A and B antigens or none)</td>
</tr>
<tr>
<td>O (has anti-A &amp; anti-B)</td>
<td>O (contains no A or B antigen)</td>
</tr>
</tbody>
</table>
DETERMINATION OF ABO BLOOD TYPE

ABO typing is required in the following situations. Some of these will be discussed in more detail later in the reading material.

- Blood donors
- Transfusion recipients
- Transplant candidates and donors
- Prenatal testing
- Newborns (sometimes)
- Paternity testing (part of the process)

The preferred specimen for ABO typing is blood collected in an EDTA (lavender or pink stopper/closure) evacuated tube. Blood specimens collected in tubes that produce serum can be used; i.e., red or gold stopper/closure evacuated tubes. However, evacuated tubes containing gel are not acceptable for ABO typing.

The evacuated tubes are centrifuged so both plasma (or serum) and blood cells can be accessed for testing. The blood cells (red) move to the bottom of the tube and the plasma/serum (clear or yellowish fluid) sits on top. Pipettes are used to suction up a small amount of red blood cells from the bottom of the tube. The red blood cells are tested for the presence or absence of the A and B antigens using antisera from manufacturers with known anti-A, anti-B, and anti-A,B.

Testing for the antigens is known as forward typing. If the manufacturers’ antisera detect the A and/or B antigen on the patient’s cells, the reaction seen is agglutination (or clumping) of the patient’s red blood cells. If no antigen is detected by the manufacturer’s antisera, there is no agglutination (this occurs with type O). If a patient’s cells agglutinate with the manufacturer anti-A antisera, that patient’s blood type is type A or AB (because both of those types contain the A antigen).

In this photograph, there are three drops of blood that have been mixed with antisera. Starting from the left, the first two drops show agglutination, which indicates an antigen:antibody reaction has occurred. The third drop is not agglutinated indicating an antigen:antibody interaction has not occurred.

A blood typing test cannot rely on the front typing alone. The laboratory must also test the patient for the presence of the appropriate naturally occurring anti-A and anti-B antibodies. This is accomplished by pipetting a couple drops of the patient’s centrifuged plasma (or serum) and testing it for agglutination with red blood cells from
manufacturers with known A and B antigens. This testing for antibodies is known as reverse typing.

The presence of agglutination is called a positive or + result. The absence of agglutination is called a negative or 0 result. The results of the four major blood groups with manufacturers’ antisera and cells are shown in the following table.

<table>
<thead>
<tr>
<th>Forward Typing (patient cells with known antisera)</th>
<th>Blood Type Interpretation</th>
<th>Reverse Typing (patient plasma with known cells)</th>
<th>Blood Type Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-A,B</td>
<td>A</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>B</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AB</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>O</td>
</tr>
</tbody>
</table>

The forward typing interpretation and reverse typing interpretation must always match. If they do not, this is called an ABO typing discrepancy and it must be resolved as the ABO blood type of the patient cannot be identified. As mentioned earlier, there are times when the A and B antigens can be ‘lost’ or ‘acquired’ on red blood cells due to certain infections or diseases. There are also times where the anti-A and anti-B antibodies can decrease in quantity so routine testing does not detect their presence. And there are times when additional antibodies can be developed that can react with the manufacturer’s red cells.

NOTES:

- The testing in the photograph on the previous page has been performed on a glass slide. In a donor center or hospital transfusion service laboratory, slide testing is not practical. ABO typing is usually performed in gel microtiter plates or small glass tubes. However, the presence or absence of agglutination is still the reaction that is observed.
- Anti-A,B antisera is used to confirm the presence of the A and B antigens. There are some A antigens that are very weak and they may not react with anti-A antisera, causing a false negative reaction and a potential mistyping of the blood. Anti-A,B is a stronger antibody and using it will almost always detect these weak A antigens.

This is a simplified discussion of the ABO system and typing. There are subgroups of A and B that can cause issues with determining the blood type, and whenever there are issues with determining a blood type, donor blood cannot be used, and a patient cannot be transfused until the blood type is definitively confirmed. There is even a rare type of blood called Bombay [as it was first identified in Bombay (now known as Mumbai), India], where the cells lack the A and B antigen, in addition to an antigen called H, which is present on all A, B, AB, and O blood types. A Bombay blood type has anti-A, anti-B, and anti-H in their plasma making them incompatible with any blood type other than another Bombay. The Bombay blood type is found in 1 of 10,000 individuals in India and 1 in a million people in Europe and other countries. People with this blood type are encouraged to donate their own blood and have it frozen for potential future transfusion.
THE Rh BLOOD GROUP SYSTEM

The second most important blood group system related to blood transfusion is the Rh blood group system. This system was named (in error) after the Rhesus monkey. This system is among the most complex blood groups known and it includes 49 antigens. The nomenclature of the system is also complex due to the discoveries of the D antibody and the D antigen by two separate groups of researchers, each using their own terminology. Eventually it was realized the discoveries of the two researchers were related and the terminology was (somewhat) simplified.

Fortunately, for routine transfusion medicine purposes, the presence or absence of only one of these antigens is of the most importance. This is the D antigen. If this antigen is present on their red blood cells, that individual is Rh positive (or D positive). If the D antigen is absent, the individual is Rh negative (or D negative).

The Rh blood group system is very different from the ABO system. Antibodies to the antigens in the Rh system are not naturally occurring. They develop only after an individual is exposed to the antigens during a blood transfusion or during pregnancy.

During pregnancy, blood cells from the fetus can enter the maternal blood circulation. If the antigens on the fetal blood cells are different from those of the mother, the mother can develop antibodies against them.

In the Rh system, the D antigen is very antigenic; i.e., very strong. Most Rh negative individuals who are exposed to the D antigen during transfusion or pregnancy will develop anti-D. Anti-D is a powerful antibody. When it is present and it encounters blood cells with the D antigen during a transfusion or pregnancy, a hemolytic reaction will result. A reaction of this type has serious consequences, therefore Rh typing for the presence or absence of the D antigen is determined on blood donors, transfusion recipients, and prenatal women.

Rh GENETICS AND THE D ANTIGEN

All the antigens in the Rh system are inherited on genes from the parents. This course will only discuss the D antigen. The D antigen is very complex and there may be as many as 30 genetic variations of it, some of which are very weak. The discussion of these variations is beyond the scope of this CE course.

Testing for the D antigen is usually performed at the same time as ABO typing. As anti-D antibodies do not naturally occur, there is no corresponding reverse typing as there is for ABO antibodies. Only manufacturers’ antisera containing anti-D is used to determine the presence or absence of the D antigen. Testing is performed using the same methodology as that used for ABO typing. Due to the complexity of the D antigen, additional typing procedures may be performed.
Whenever possible, individuals who are Rh negative should receive Rh negative blood when transfusion is required to prevent the development of anti-D antibodies. Anti-D is in the IgG classification of antibodies. This is especially important in women of child-bearing age as the development of anti-D can result in hemolytic disease of the newborn, which is potentially fatal. This will be discussed later in the reading material.

However, in extreme trauma situations it may be necessary to transfuse Rh negative people with Rh positive blood. This is an acceptable practice as long as the patient does not already have anti-D antibodies. In situations such as this it is necessary to save the patient’s life and worry about the development of anti-D antibodies later.

**ABO AND RH BLOOD TYPES AMONG POPULATIONS**

It is interesting to note that ABO and Rh blood types vary among populations, races, and ethnic groups. The mix of the different blood types in the U.S. population is shown in the table below. Note that approximately 85% of the U.S. population is Rh positive (D antigen positive).

<table>
<thead>
<tr>
<th></th>
<th>African-American</th>
<th>Asian</th>
<th>Caucasian</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>O +</td>
<td>47%</td>
<td>39%</td>
<td>37%</td>
<td>53%</td>
</tr>
<tr>
<td>A +</td>
<td>24%</td>
<td>27%</td>
<td>33%</td>
<td>29%</td>
</tr>
<tr>
<td>B +</td>
<td>18%</td>
<td>25%</td>
<td>9%</td>
<td>9%</td>
</tr>
<tr>
<td>AB +</td>
<td>4%</td>
<td>7%</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>O -</td>
<td>4%</td>
<td>1%</td>
<td>8%</td>
<td>4%</td>
</tr>
<tr>
<td>A -</td>
<td>2%</td>
<td>0.5%</td>
<td>7%</td>
<td>2%</td>
</tr>
<tr>
<td>B -</td>
<td>1%</td>
<td>0.4%</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td>AB -</td>
<td>0.3%</td>
<td>0.1%</td>
<td>1%</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

The following table identifies the percentages of ABO and Rh types in other countries.

<table>
<thead>
<tr>
<th></th>
<th>Australia</th>
<th>India</th>
<th>Israel</th>
<th>Japan</th>
<th>Philippines</th>
<th>South Africa</th>
<th>Saudi Arabia</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td>O +</td>
<td>40%</td>
<td>35.1%</td>
<td>32%</td>
<td>29.9%</td>
<td>45%</td>
<td>39%</td>
<td>48%</td>
<td>32%</td>
</tr>
<tr>
<td>A +</td>
<td>31%</td>
<td>21.7%</td>
<td>34%</td>
<td>39.8%</td>
<td>22.5%</td>
<td>32%</td>
<td>24%</td>
<td>37%</td>
</tr>
<tr>
<td>B +</td>
<td>8%</td>
<td>30.5%</td>
<td>17%</td>
<td>19.9%</td>
<td>24.5%</td>
<td>12%</td>
<td>17%</td>
<td>10%</td>
</tr>
<tr>
<td>AB +</td>
<td>2%</td>
<td>7.3%</td>
<td>7%</td>
<td>9.9%</td>
<td>5%</td>
<td>3%</td>
<td>4%</td>
<td>5%</td>
</tr>
<tr>
<td>O -</td>
<td>9%</td>
<td>2%</td>
<td>3%</td>
<td>0.15%</td>
<td>&lt;1%</td>
<td>7%</td>
<td>4%</td>
<td>3.9%</td>
</tr>
<tr>
<td>A -</td>
<td>7%</td>
<td>1.2%</td>
<td>4%</td>
<td>0.2%</td>
<td>&lt;1%</td>
<td>5%</td>
<td>2%</td>
<td>7%</td>
</tr>
<tr>
<td>B -</td>
<td>2%</td>
<td>1.7%</td>
<td>2%</td>
<td>0.1%</td>
<td>&lt;1%</td>
<td>2%</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>AB -</td>
<td>1%</td>
<td>0.4%</td>
<td>1%</td>
<td>0.05%</td>
<td>&lt;1%</td>
<td>1%</td>
<td>0.3%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Worldwide, O Positive (O+) is the most common blood type and AB Negative (AB-) is the least common blood type.
OTHER BLOOD GROUP SYSTEMS

While the ABO and Rh blood group systems are the most important systems related to blood transfusion, there are 33 other blood group systems with approximately 300 antigens identified. People can develop antibodies to these antigens as a result of transfusion and/or pregnancy. Some of the antibodies are considered clinically significant; i.e., capable of causing hemolytic transfusion reactions and hemolytic disease of the newborn. Both of these conditions will be discussed later. Other antibodies are not considered to be clinically significant.

The names of some of these blood group systems follow.

<table>
<thead>
<tr>
<th>Kell</th>
<th>Kidd</th>
<th>Duffy</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Lewis</td>
<td>Cartwright</td>
</tr>
<tr>
<td>Lutheran</td>
<td>I/i</td>
<td>Diego</td>
</tr>
<tr>
<td>MNS</td>
<td>Colton</td>
<td>Dombrock</td>
</tr>
</tbody>
</table>

When antibodies to blood group systems outside the ABO system are present, they are called unexpected, irregular, or atypical. For the purposes of this CE course, they will be called unexpected antibodies. The purpose of pretransfusion testing, which includes antibody screening and compatibility tests, is to detect the presence of unexpected antibodies. If an antibody screening test does detect an unexpected antibody, antibody identification panel tests are performed to determine the specific antibody or antibodies that are present. Antibody identification is not always easy, and it may take several hours to identify what specific antibody or antibodies are present.

When a patient has an antibody or antibodies, all units of blood transfused must be negative for the offending antigen or antigens. The presence of an antibody or a combination of antibodies may make it difficult to locate compatible blood for transfusion. For example, an antigen in the Kell blood group system called Cellano, abbreviated ‘k’, is found on 91% of Caucasians and 98% of African Americans in the United States. Therefore, if a patient has an anti-k antibody, it will be difficult to find compatible blood as only 9% of Caucasians and 2% of African Americans lack the k antigen.

Blood donor centers type donor units for antigens in other blood group systems and sequester the units with rarer blood types so they can be available for patients with rare antibodies or combinations of antibodies. In some circumstances, donor units with rare types are frozen in a process that preserves the red cells for at least 5 years. When needed, the blood is thawed and used for transfusion.

OTHER TRANSFUSION-RELATED LABORATORY TESTING

Donor centers and hospital blood bank labs perform testing other than ABO and Rh typing. Following is a brief discussion of a few of the most common procedures.

1. **Antibody Screen Test**: All blood donors, all prenatal women, and patients being tested prior to blood transfusion are screened for the presence of antibodies to systems other than the ABO system. As mentioned above, these antibodies are
called 'unexpected' to distinguish them from ABO antibodies, which are regularly seen and expected to be present.

This is called an antibody screen test. It may be ordered with the blood type as a type and screen test. This test will detect the presence of most of the clinically significant antibodies. The antibody screen test is also known as an indirect antiglobulin or indirect Coombs test. The term 'indirect antiglobulin' refers to the method by which most clinically significant antibodies can be detected; 'Coombs' is the name of the researcher who developed the procedural method.

2. **Antibody Identification Panel**: If the antibody screening test is positive, the antibody must be identified by performing an antibody identification panel test. This testing may become involved and take several hours. It may not take much time to identify a single common antibody; however, identifying unusual antibodies and/or combinations of antibodies may be time-consuming.

3. **Direct Antiglobulin Test (DAT)**: This test identifies the presence of antibodies attached to red cells in the circulation of a patient. If the DAT is positive, this is an abnormal finding. It can indicate an autoimmune hemolytic anemia, a hemolytic transfusion reaction, or hemolytic disease of the newborn. This test is also known as a Direct Coombs Test.

4. **Crossmatch**: Before a transfusion of red blood cells, a crossmatch test is performed. A crossmatch may be referred to as a type and cross, or shortened to XM. In this test, red blood cells from the potential donor unit are added to plasma from the intended transfusion recipient (patient). If agglutination occurs, an incompatibility is present. The purpose of a crossmatch is to prevent the transfusion of incompatible cells. In most instances, a crossmatch verifies the donor cells are ABO compatible with the recipient. However, a crossmatch will not do the following.

   a. Detect all unexpected antibodies in the recipient.
   b. Detect all ABO typing errors in either the donor or recipient.
   c. Detect most Rh (D) typing errors in the donor or recipient.
   d. Prevent all types of transfusion reactions.

The specimen of choice for the above tests is the same as that for ABO and Rh typing: blood collected in a lavender or pink EDTA evacuated tube.

**BLOOD DONATION**

**COLLECTION OF DONOR UNITS**

Blood donation is a simple and safe process that takes 45-60 minutes. To be blood donors, individuals must meet the requirements, pass the pre-donation screening questions, and pass the short health exam (pulse, temperature, blood pressure, and hemoglobin test). Potential blood donors must be 18 years of age or older, weigh at least 110 pounds, and be in general good health. In some instances, 16- and 17-year-olds may donate when a form is signed by a parent or guardian. Potential donors
should be well-hydrated and have eaten a well-balanced meal within four hours of the donation.

Because of potential transmission of certain diseases, or potential harm to the individual, some people are not allowed to donate blood. These include but are not limited to individuals with the following diseases, disorders, travel history, or personal history.

- HIV/AIDS
- Hematological cancer such as leukemia, lymphoma, or Hodgkin disease
- Kidney, lung, or liver failure
- Recreational drug use by injection
- Visited or lived in England, Scotland, Wales, Northern Ireland, Isle of Man, Channel Islands, Gibraltar or Falkland Islands for a total of 3 months or more from 1980-1996
- Spent a cumulative of 5 or more years since 1980 in European countries
- Men or women who have engaged in sex for money or drugs since 1977

Individuals wanting to donate blood may be temporarily deferred (days-years) for travel to certain countries, certain diseases (cardiac, syphilis, gonorrhea, anemia), vaccinations, and some medications. The latest FDA guidance recommends that men who have had past sex with other men are no longer indefinitely deferred; they are instead temporarily deferred for 12 months after the most recent sexual contact with another man.

**Single Unit Blood Donations**

The majority of blood donations are single units. Collection of a unit of whole blood uses a plastic bag with a needle attached. The bag has smaller bags called ‘satellite bags’ attached to it. The satellite bags are used to for preparation of components after the donor blood is collected. Blood components, which were previously mentioned, will be discussed in more detail later in the reading material.

Blood donor bags are sterile and contain an anticoagulant solution and a preservative solution. The anticoagulant prevents the collected whole blood from clotting, and the preservative solution provides nutrients for the red blood cells to utilize for their
metabolism during the storage period. The blood contains living red cells and the cells must be provided with suitable nutrients to continue to live so they can function when transfused.

The single unit whole blood donation process takes about 15 minutes. The antecubital area of the arm is cleaned with an antiseptic, and once the antiseptic has dried, a puncture is made using a 16 gauge needle. Approximately 450 mL of whole blood is collected in the bag. When the donation is complete, the tubing is clamped twice and cut between the clamps. On the donor side of the clamp, the clamp is released and blood is collected into evacuated tubes, and the needle is removed. The donor elevates his/her arm and applies slight pressure to promote clotting of the venipuncture site. The unit of blood is permanently clamped and the bag, evacuated tubes, and donor records are labeled with identical bar code labels to enable tracking of the donation and follow up testing.

When the donor’s collection site has stopped bleeding, a pressure bandage consisting of folded gauze and a self-adherent flexible wrap such as Coban™ is applied. The donor is taken to the recovery area where juice and snacks are provided. Donors stay in this area for about 15 minutes, and then they are able to leave the donation facility.

Individuals who meet the requirements can donate a unit of whole blood every eight weeks.

Apheresis Collection

Red blood cells and blood components can also be collected through an automated process called apheresis. During apheresis, donors can selectively donate only the blood components that are needed most, and the remaining portions of the blood are returned to them. By using apheresis, a single donor can donate only platelets, only plasma, or two units of red blood cells. Apheresis is used most frequently for platelet collection and double red blood cell collection.

The apheresis processes takes longer than a single whole blood donation. Depending on the blood component collected, it may take up to two hours. During apheresis, the donor usually has a needle in one arm removing whole blood and the whole blood is moved into the apheresis instrument. The apheresis machine centrifuges the whole blood into red blood cells, platelets, and plasma. The portions of the whole blood that are not being collected are returned to the donor. Some apheresis collections use a technique where the donor has needles in both arms, one removing blood which enters the apheresis machine, and the other arm receiving the components of blood not needed from the apheresis machine. The frequency of apheresis collections depends on the blood component being collected.

Following is an excerpt from the Armed Services Blood Program website (http://www.militaryblood.dod.mil/) describing apheresis donation of platelets. The photograph identifies an individual undergoing an apheresis procedure for platelet collection and an apheresis machine.
From Whole Blood to Platelets
12/26/2012
By Jerrick Alexander, ASBP Blood Donor Recruiter, Pentagon Blood Donor Center

Jill Shanteau from the Department of the Army Headquarters at the Pentagon has been donating whole blood to the Armed Services Blood Program since 2011. When she recently looked at the status boards showing which types of blood were currently needed for whole blood donations at the Pentagon Blood Center, she thought that the program didn’t want her blood. When she asked why, staff members at the donor center explained that her blood type was better suited for platelet donations. Shanteau then scheduled her next appointment for platelets.

Shanteau said she prepared for her donation by drinking plenty of water and eating a bowl of oatmeal and other iron-rich food the day of her donation. When she came into the blood donor center for her first platelet donation, she was motivated and excited to roll up her sleeve and save lives.

As she laid down for the procedure, staff at the Pentagon Blood Donor Center prepared her movie and wrapped her up in a warm blanket. All warm and cozy, and ready for her donation, Shanteau said, “I could get used to all of this attention!”

Although Shanteau may have started off donating whole blood, she said she is now going to become a dedicated platelet donor.

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AFTER BLOOD DONATION

When a blood donation is complete, two processes occur simultaneously.

- The unit of blood is centrifuged to separate it into components: red blood cells, platelets, and plasma. The platelets and plasma are removed into the satellite bags. The red blood cells stay in the primary collection bag. All bags are labeled with the same bar code label. The three blood components are placed in quarantine in the appropriate storage refrigerators/freezers.
  - Red blood cells are stored at 1-6°C
  - Plasma is frozen and stored at ≤ -18°C
  - Platelets are stored at 20°-24°C with continuous gentle agitation

If apheresis products are collected, they are sent directly to the quarantine storage refrigerators/freezers.

- The evacuated tubes collected from the blood donor are tested for the following.
  - ABO and Rh type
  - Antibody screen and if positive, antibody identification
Infectious disease testing for the following bloodborne pathogens:

- Trypanosoma cruzi
- Hepatitis B virus
- Hepatitis C virus
- Human Immunodeficiency Virus, Types 1 and 2
- Human T-Lymphotropic Virus
- Syphilis
- West Nile Virus
- Zika Virus (as of September 2016)

If an infectious disease test is positive, all components are disposed and not released for transfusion. The donor is notified of the test results. The test results are confidential and are shared only with the donor, except as may be required by law.

Donor blood that has irregular antibodies must also be discarded due to the risk of transfusion reaction if the recipient has the offending antigen on their cells.

BLOOD COMPONENTS

With very few exceptions, all whole blood donations are fractionated into blood components. The most common blood components available for transfusion include red blood cells, plasma, platelets, and cryoprecipitate. These components are prepared in the donor facility where the blood is collected.

Commercial manufacturers can further fractionate components of plasma into derivatives such as Factor VIII; Factor IX; Rh immune globulin; other immune globulins; and albumin and plasma protein fraction.

Clinical indications for the transfusion of blood components and administration of commercially manufactured fractions will be discussed later in the reading material.

SPECIMEN COLLECTION FOR LABORATORY TESTING

SPECIMEN REQUIREMENTS

As previously mentioned, the specimen of choice for transfusion services testing is blood collected in a 7 mL EDTA-anticoagulated purple or pink closure evacuated tube (red cells/plasma). The only difference between these two evacuated tubes is the information to be completed on the tube label. For patient safety, specimens collected for blood bank testing have specific labeling requirements which are discussed below.

Plain red closure tubes (red cell clot/serum, 10 mL) are also acceptable for testing. However, EDTA tubes are preferred over plain red tubes for the following reasons.

- EDTA tubes can be centrifuged immediately upon receipt in the laboratory; plain red closure tubes must be allowed to clot for at least 20-30 minutes before
centrifugation. In emergency situations, valuable time is saved using EDTA tubes.
- The plasma obtained from EDTA tubes is unlikely to contain fibrin which is problematic when performing patient testing. Fibrin can interfere with some blood bank lab testing. Plain red closure tubes often contain fibrin.

Notes:
- These are recommended specimen requirements; laboratories may determine their own sample requirements.
- If patients have been transfused in the past or are/have been pregnant, and transfusion is anticipated or scheduled, specimens must be collected no sooner than three days prior to the transfusion. The day of collection is considered Day 0; the specimen can be used for additional testing until 11:59 PM Day 3.
  - New specimens are required every three days as an unexpected antibody from the previous transfusion or pregnancy could develop within this time period.
  - Many healthcare facilities use the three day limit for all pretransfusion testing as it is easier to have the same requirements for all specimens, and patient history information may be unreliable.

PATIENT IDENTIFICATION/SPECIMEN LABELING

Proper patient and specimen identification are critically important for blood bank testing. Errors in patient and specimen identification are called clerical errors, and these are the most common cause of fatal and near-fatal transfusion reactions. Blood bank laboratories will reject specimens that are not labeled exactly following their standard operating procedures.

For accurate patient identification, the computer-generated or manually prepared blood bank testing request must include the following:

- Patient’s first and last name; middle initial or name is preferred
- Facility patient identification number such as a medical record number
- Date of birth
- As needed other information may be included such as
  - Account/billing number
  - Gender
  - Location
  - Date
  - ICD-10 Code to establish medical necessity
  - Physician name

Patients must wear a hospital identification band that includes the following information. Additional information may be present on the identification band.

- Patient’s first and last name; middle initial or name is preferred
- Facility patient identification number such as a medical record number
- Date of birth
The patient must be positively identified before collection of the blood sample. This is done by comparing at least two unique identifiers (such as the patient’s first/last name/middle initial and facility identification number/medical record number) on the blood bank test requisition with the information on the patient’s identification band. This information should be confirmed by asking the patient to state his/her name and birth date, i.e., “What is your name and birth date?”* If the patient is unable to respond, rely on the arm band identification.

*Do not ask “Are you John/Jane Doe?” Many patients may simply respond “Yes” as he/she may have difficulty hearing, want to cooperate, or simply may not understand what is being asked.

All patient identification information must match exactly or the specimen cannot be collected for testing. All discrepancies, no matter how insignificant they might seem, must be resolved before a specimen can be collected for testing.

Immediately following specimen collection, the evacuated tubes must be labeled at the bedside. The tube labels must include the following information.

- Patient’s first and last name; middle initial or name if included on the testing requisition
- Facility identification number/medical record number
- Date and time of collection
- Specimen collector’s identification (initials or unique code number); Note: While it is good practice to have the specimen collector’s identification on all tubes of blood collected for laboratory testing, it is required for transfusion medicine testing. Other laboratory departments may accept blood tubes without collector identification on the label, if this information is on a requisition or in the information system. For transfusion medicine testing, it must be on the tube label.

Some blood banks will accept labels that are computer-generated with handwritten date, time, and specimen collector’s identification. Other blood banks will accept entirely handwritten labels only.

Blood bank laboratories must maintain logs of all individuals who collect specimens for blood bank testing. The logs must include each individual’s name and initials or unique code number. It is absolutely necessary that a specimen submitted for testing can be traced back to the individual who collected the specimen.

Many healthcare facilities now utilize barcode systems to assist with patient identification for specimen collection, medication administration, updating of medical records, blood transfusion, and more. A unique barcode is printed on the patient’s identification band, and handheld scanners are used to confirm the patient’s identity before specimen collection, blood transfusion, medication administration, etc.

Some barcode systems print tube labels in a central location that are added to the tube at the bedside; other systems have portable printers that print tube labels at the bedside only after scanning the barcode on the patient’s identification band.

Following are pictures of one manufacturer’s barcoded identification band system. The scannable band comes with labels that are added to the specimen tubes and blood products to be transfused, as well as the blood bank testing in the laboratory, and the
nursing documentation for transfusion. With a system such as this, the patient, the blood products, and the paperwork are all linked to assure safer transfusions.

Some healthcare facilities utilize a second patient identification banding system to provide an additional ‘layer’ of safety for patient identification for blood bank testing. Prior to collecting specimens for blood bank testing, and after confirming the patient’s identification, another identification band is placed on the patient. Typically, these bands have a place on which the specimen collector handwrites the patient’s name, facility identification/medical record number, date/time, and his/her unique identification (initials/code number). This section is peeled off the band, and is placed on the specimen tube. Where the label was removed, the handwritten information is carbon-copied on the band.

The identification band has a unique alphanumeric code, and a series of small stickers with this code. The code is also on the specimen collection tube label. Some of the small stickers are cut off the band and taken to the blood bank laboratory with the specimen tube. In the laboratory, they are used to identify patient testing records. When it is time for blood transfusion, the patient’s name, facility identification/medical record number, and the unique blood bank band alphanumeric code are used to identify the patient.
The picture below shows an example of a banding system that is used for pre-transfusion and transfusion identification.

![Securline® Blood Band Commercial Banding System for Identification of Blood Recipients](image)

Reprinted with permission of Precision Dynamics Corporation, San Fernando, CA

**EMERGENCY SITUATIONS**

In emergency situations when the identification of the patient cannot be made, an established emergency identification procedure must be used. When names and birthdates are not known, an alternative naming system can be used such as John Doe, Jane Doe, Male Trauma Patient 1, Female Trauma Patient 2, etc. Most Emergency Departments have trauma packets prepared that contain hospital identification bands with preassigned medical record numbers and temporary gender-specific trauma identification. The packet contains everything needed to complete the registration of the patient quickly, collect needed specimens for blood bank testing, and assure safe transfusion practices.

**IMPORTANCE OF PATIENT IDENTIFICATION**

There is zero tolerance for errors in positive identification of transfusion recipients. The transfusion of blood components to the incorrect recipient may result in a deadly transfusion accident. It is imperative that phlebotomists and other healthcare professionals responsible for phlebotomy approach specimen collection for blood bank testing with the utmost of caution as they have a life and death role in this process. Failure to provide adequate concentration and attention to this task endangers the life of the patient.

**Clerical Errors**

The majority of fatal transfusion reactions result from transfusion of ABO incompatible blood. These transfusion-related deaths are almost always due to clerical errors. The potential for clerical error begins with the individual placing the identification band on the patient, the phlebotomist or other healthcare professional collecting the blood specimen, and extends through the entire testing process, the release of the blood product from the blood bank, the identification of the patient by the nurse prior to the transfusion, and the transfusion itself. Each step in the process provides opportunities for error. Careful adherence to all procedures and protocols reduces the likelihood of clerical error.
In 2012 the FDA reported three fatalities occurred due to ABO-incompatible transfusions. Two of these were due to clerical error and these are described below.

- Fatality 1: Phlebotomist collected extra specimens on two patients, anticipating future crossmatch orders for both. These extra specimens were correctly labeled. After receiving crossmatch orders, the phlebotomist switched the specimens and applied the blood bank labels over the existing labels, before delivering the specimens to the Blood Bank for testing. As a result, the incorrect blood groups were assigned to both patients, and the group O patient was transfused with an incompatible group A red blood cell unit.

<table>
<thead>
<tr>
<th>Patient X (really Group O)</th>
<th>Patient Y (really Group A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood labeled as Patient X is typed as Group A (really contains Patient Y blood)</td>
<td>Blood labeled as Patient Y is typed as Group O (really contains Patient X blood)</td>
</tr>
<tr>
<td>Patient X received blood transfusion of group A blood</td>
<td>No transfusion occurred. However, Group O red cells would be compatible with a Group A person</td>
</tr>
<tr>
<td>Patient X’s anti-A antibodies seek out and destroy the transfused A red cells</td>
<td></td>
</tr>
<tr>
<td>Patient dies from incompatible blood transfusion</td>
<td></td>
</tr>
</tbody>
</table>

This is a classic example of what is known as ‘wrong blood in tube’ or WBIT. The specimen appears to be labeled correctly for the patient, but the blood in the tube is from a different patient. This error can also occur when a specimen is mislabeled (collected on the right person but labeled as a different patient). This example also demonstrates the importance of labeling specimens at the bedside.

- Fatality 2: A Group AB red blood cell unit, correctly labeled for the intended patient, was issued to the Emergency Department (ER), and transfused to another patient in the ER who was a Group A. The anti-B in the group A patient destroyed the transfused red cells when it reacted with the B antigen on the cells. This error occurred due to failure of the ER personnel to properly identify the patient prior to transfusion.

<table>
<thead>
<tr>
<th>ER Patient R</th>
<th>ER Patient S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient identified correctly by phlebotomist; phlebotomist correctly labels blood specimen</td>
<td>Patient identified correctly by phlebotomist; phlebotomist correctly labels blood specimen</td>
</tr>
<tr>
<td>Blood bank personnel correctly perform compatibility testing and select compatible units for transfusion; patient is Group AB</td>
<td>Blood bank personnel correctly perform compatibility testing and select compatible units for transfusion; patient is Group A</td>
</tr>
<tr>
<td>ER Nurse picks up red blood cell unit for transfusion to ER Patient R</td>
<td>ER Patient S receives unit of red blood cells meant for ER Patient R</td>
</tr>
</tbody>
</table>
However, ER Patient R does not receive blood transfusion meant for him

ER Patient S anti-B antibodies seek out and destroy the B antigens on the Group AB blood

ER Patient S dies from incompatible blood transfusion

The third ABO incompatible transfusion that resulted in death in 2012 was not related to a clerical error. It resulted when a Group A patient received Group O apheresis platelets from two donors who had very high titers of anti-A. The anti-A in the donor platelets attached the patient’s A antigen on the red blood cells, resulting in hemolysis that lead to the patient dying.

Fatal ABO hemolytic transfusion reactions were reported by the FDA in 2013 (1), 2014 (4), 2015 (2), and 2016 (4). Around half of these were the result of very similar clerical errors as the ones described above. Each incident involved a failure to properly identify the recipient prior to the transfusion.

To avoid WBIT ABO-incompatible transfusions, some facilities require two separate blood type results on file from two independent blood collections for each patient prior to transfusions (except in cases of emergency). In the event that a patient has no blood type history on file in a facility, two separate blood draws at different times by different specimen collectors reduces the risk of a patient identification error.

**BLOOD COMPONENTS AND INDICATION FOR TRANSFUSION**

**RED BLOOD CELLS**

Red blood cells are transfused to people with anemia due to diseases and conditions, or sudden loss of blood from trauma, hemorrhage, or surgery. The purpose of red blood cell transfusion is to provide red cells to transport oxygen to organs and tissues. When stored at 1-6°C, a unit of red blood cells has an expiration date of 42 days.

Transfusion of red blood cells is usually recommended when a patient’s hemoglobin level is 7.0 g/dL, and he/she is showing clinical symptoms such as shortness of breath, syncope, etc. [The normal reference ranges (per Mayo Medical Laboratory*) for adult hemoglobin is 13.5-17.0 g/dL (male) and 12.0-15.5 g/dL (female)]. One to two units of red blood cells are recommended for transfusion, and a post-transfusion hemoglobin level can be measured as soon as 15 minutes after the end of the transfusion, as long as the patient is not actively bleeding. Each unit transfused should increase the patient’s hemoglobin level by 1 gm/dL. More units of red blood cells may be needed for transfusion if the patient is still symptomatic or still bleeding.

*Reference ranges vary slightly from laboratory to laboratory as they are dependent on the patient population and the method used to perform the test.

Red blood cell transfusions must be ABO compatible, and should be Rh compatible in all but the most emergent of situations. If a patient has developed anti-D antibodies, red blood cells must be ABO and Rh compatible.
PLASMA

When frozen shortly after removal from red blood cells, plasma (now called fresh frozen plasma or FFP) contains many important coagulation factors. FFP serves as a transfusion source of unconcentrated coagulation factors without platelets. When frozen, FFP has an expiration date of one (1) year. Prior to transfusion, it must be thawed, and after thawing, the expiration date is 24 hours when stored at temperatures of 1-6°C.

If the plasma is not frozen within a certain period of time after the collection of the donor unit, the coagulation factors greatly decrease in their ability to function. Freezing the plasma allows the coagulation factor life span to remain stable. Once thawed, the usefulness of the coagulation factors decreases somewhat after 24 hours. However, for most patients, plasma that has been thawed for more than 24 hours still has therapeutic value and can be transfused. Many facilities store thawed plasma for up to five days at refrigerated temperatures (1°-6°C). The product, now labeled as “thawed plasma,” is beneficial to keep in inventory when thawed plasma is needed quickly for emergency situations, such as trauma or unexpected blood loss during surgery.

Plasma is recommended for transfusion when people have lost large quantities of blood from surgery, hemorrhage, or trauma, or in certain disease states.

- When patients lose large quantities of blood, they also lose blood coagulation factors. Patients are then unable to stop bleeding, which only worsens their condition. Therefore, transfusions of units of thawed FFP provide a temporary source of coagulation factors until patients can manufacture more of their own. Patients in this state will also receive red blood cell and platelet transfusions.

- Other indications for FFP transfusion are liver disease and disseminated intravascular coagulation (DIC).
  o The liver makes many of the coagulation factors. When liver failure occurs, production of these coagulator factors declines or stops. FFP transfusions can help provide coagulation factors in these patients if bleeding problems begin.
  o In DIC, patients begin to produce small blood clots throughout their bodies. In doing this, they use up their coagulation factors and they spontaneously begin to bleed. This is a unique problem as they suffer the effects of both blood clots and spontaneously bleeding. Transfusion of FFP provides a temporary source of coagulation factors until the cause of the blood clot production can be treated.

Typically, 2-4 units of FFP are transfused. To measure the effectiveness of FFP transfusions, PT/INR and aPTT tests are performed. If the first transfusions of FFP have not brought the patient into the reference range for these tests, more FFP may be given.

FFP transfusions must be ABO compatible. While FFP does not contain red cells, it contains ABO antibodies. Therefore, the ABO antibodies in the FFP must be compatible with the red cell antigens of the transfusion recipient.
PLATELETS

Platelets are microscopic particles present in the circulation that assist with blood coagulation. There are many reasons that people develop thrombocytopenia including but not limited to the following.

- Massive blood loss from trauma, hemorrhage, or surgery
- Chemotherapy treatment
- Platelet function disorders
- Platelet production disorders
- DIC

The reference range for a platelet count is 150,000-450,000 /µL (Mayo Medical Laboratories). Generally, platelet transfusions are indicated in the following situations.

- Patients with massive blood loss from trauma, hemorrhage, or surgery
- Patients having surgery (other than ophthalmic or neurosurgery) with platelet counts of 50,000/µL or less
- Ophthalmic and neurosurgery patients with platelets counts of <100,000/µL
- Patients with counts of < 10,000/µL to prevent spontaneous bleeding
- Patients with platelet function/production disorders who have active platelet-related bleeding

Platelets are transfused as 6 individual donor units pooled together, or one apheresis platelet collection. This transfusion should raise the platelet count by 30,000-60,000/µL.

Platelets are stored at room temperature and must be gently and continuously agitated during storage. Platelets have a 5 day expiration date from the date of the donation. After single donor platelets have been pooled together for transfusion, the expiration date is 4 hours.

As with FFP, platelet transfusions must be ABO compatible. While platelet transfusions contain very few red cells, they do contain donor antibodies. Therefore, the ABO antibodies in the platelets must be compatible with the red cell antigens of the transfusion recipient. Rh type does not need to be considered for transfusion of platelets. However, as platelet preparations may contain some red blood cells, some patients may develop antibodies from repeated platelet transfusions. As an example, Rh negative recipients may develop anti-D after transfusion of numerous units of Rh positive platelets.

CRYOPRECIPITATE

Donor centers maintain some units of FFP from which they prepare cryoprecipitate. Cryoprecipitate is a substance recovered from thawed FFP that contains the coagulation factors fibrinogen, von Willebrand’s factor, Factor VIII, Factor XIII, and fibronectin.
After it is prepared at the donor center, cryoprecipitate is frozen and it has an expiration date of 1 year from the donor collection date. As with FFP, it must be thawed prior to transfusion.

Transfusion of cryoprecipitate is most often indicated to control bleeding in people with von Willebrand’s disease, Factor XIII deficiency, and low levels of fibrinogen. These patients may not bleed spontaneously, but may need assistance with coagulation following surgical procedures, including minor ones such as tooth extractions. Cryoprecipitate is generally transfused as 4-10 donor units pooled together. After thawing, cryoprecipitate has an expiration date of 6 hours and is stored at room temperature; after pooling, the expiration date is 4 hours and pooled cryoprecipitate is also stored at room temperature. ABO compatibility is preferred but Rh type does not be considered when transfusing cryoprecipitate.

Cryoprecipitate may be combined with thrombin and calcium to form a ‘glue’ or sealant used in a variety of surgical situations. The fibrin glue can be directly applied to a surgical site to reduce bleeding. The glue simulates the final stage of the coagulation cascade.

**COMPATIBILITY OF PLASMA, PLATELETS, AND CRYOPRECIPITATE**

Compatibility of plasma, platelets, and cryoprecipitate transfusions is based on donor antibodies and recipient cells (transfusion of red blood cells is the opposite). Antibodies reside in plasma, and both platelets and cryoprecipitate contain amounts of plasma. Thus, consideration must be made concerning donor antibodies when determining compatibility of plasma products for transfusion.

As previously stated, with red blood cell transfusions type O is considered the universal donor and type AB is the universal recipient. With plasma products, the opposite is true: type AB is the universal donor (does not contain naturally occurring anti-A or anti-B antibodies). Type AB plasma is used in many trauma cases (when blood type is not yet known) and in neonatal transfusions.

In the case of platelet and cryoprecipitate transfusions, ABO compatibility is preferred but not always possible in emergency situations.

The table below lists ABO compatibility for transfusion of plasma, platelets, and cryoprecipitate.

<table>
<thead>
<tr>
<th>Recipient blood type</th>
<th>Compatible plasma, platelet, and cryoprecipitate types</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A, AB</td>
</tr>
<tr>
<td>B</td>
<td>B, AB</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
</tr>
<tr>
<td>O</td>
<td>A, B, AB, or O</td>
</tr>
</tbody>
</table>
SUMMARY OF ABO COMPATIBILITY FOR ALL TRANSFUSIONS

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>REQUIREMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>ABO compatible with recipient’s plasma</td>
</tr>
<tr>
<td>Fresh frozen plasma</td>
<td>ABO compatible with recipient’s red blood cells</td>
</tr>
<tr>
<td>Platelets</td>
<td>ABO compatible with the recipient’s red blood cells is preferred; emergency situations may occur where this is not possible</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>ABO compatible with recipient’s red blood cells is preferred; emergency situations may occur where this is not possible</td>
</tr>
</tbody>
</table>

PLASMA DERIVATIVES

Immune Globulins

Rh Immune Globulin

Rh Immune globulin (RHIG) is a sterile solution that contains anti-D antibodies. It is made from the pooled plasma of healthy people who have developed anti-D through pregnancy or transfusion. All individuals are tested for hepatitis C, hepatitis B, parvovirus 19, and HIV; all tests must be negative for their plasma to be used. The solution is highly processed, passed through a viral clearance filter, and it is not manufactured with thimerosal.

RHIG is given to Rh negative (D negative) women during a pregnancy to prevent the development of anti-D antibodies. During a pregnancy, it is not unusual for some of the fetus’s blood to enter the mother’s circulation. If the fetus’s blood is Rh positive (D positive), the mother can develop anti-D antibodies. These antibodies can attack and destroy the Rh positive blood of the fetus, which leads to anemia, and often heart failure, of the fetus. Rh immune globulin is administered (usually via intramuscular injection) during the pregnancy. The anti-D antibodies seek out and destroy any Rh positive blood cells they find before the mother can develop anti-D of her own. The small amount of antibodies in the Rh immune globulin does not hurt the fetus’s red cells. Common brand names of Rh immune globulin in the United States are BayRho-D, HyperRHO-S/D, RhoGam, Rhophylac, and WinRho SDF.

When the mother delivers the baby, the blood type of the baby is determined. If the baby is Rh positive, the mother receives another injection of Rh immune globulin for further protection against the development of anti-D antibodies. If the baby is Rh negative, there is no need for another injection of Rh immune globulin. Rh immune globulin should also be administered to Rh negative women who have abortions, miscarriages, amniocentesis, or any other time a fetal-maternal hemorrhage is suspected.

More than one injection of RHIG may be indicated if the fetal-maternal hemorrhage is severe enough. When indicated, a test called Kleihauer–Betke can be ordered by the physician and performed by the laboratory. This test is performed on a sample of blood from the mother. The test determines the amount of fetal cells in the mother’s bloodstream and the number of RHIG injections required to protect against stimulation of the mother’s immune system against the D-positive cells of the fetus.
When an Rh positive fetus or newborn is affected by maternal anti-D antibodies this is called hemolytic disease of the newborn or HDN. HDN will be discussed later in the reading material.

Other Immune Globulins

Immune Globulin: Immune globulin preparations, prepared from pools of human plasma, can protect against certain diseases. In the United States, only plasma that is negative for hepatitis B, hepatitis C, and HIV is used to manufacture immune globulins. These solutions contain concentrated antibodies that can provide temporary protection against bacterial or viral infections in individuals with certain autoimmune and immunodeficiency disorders including but not limited to chronic B-cell lymphocytic leukemia, Guillain-Barré syndrome, and severe combined immunodeficiency disorder.

Immune globulin is administered intravenously for prophylaxis and after potential or known exposure to severe bacterial and viral infections. This is a type of passive immunization, and it provides only temporary protection.

Hyperimmune Globulin: Hyperimmune globulin is prepared from the plasma of individuals with high quantities of antibody (or anti-toxin) against a specific organism or antigen such as hepatitis A, rabies, or rubella. In the United States hyperimmune globulin is available for the following infectious diseases.

- Hepatitis B virus
- Cytomegalovirus
- Rabies
- Respiratory syncytial virus
- Vaccinia virus
- Varicella-zoster virus
- Botulinum
- Diphtheria
- Tetanus
- Rubella

Hyperimmune globulin is administered to prevent serious life-threatening illness or serious complications in individuals who have been exposed to disease to which they are not immune, or that they are not able to produce antibodies to protect themselves. Examples of situations follow.

- The rubella virus can cause serious birth defects if a woman is exposed during the 1st trimester of pregnancy. If a woman has not been immunized and has been exposed to the rubella virus, hyperimmune rubella globulin can be administered as a way to protect the fetus against birth defects.
- An individual who has not been vaccinated with a rabies vaccine, and has been bitten by a bat confirmed to have rabies, can be treated with rabies immune globulin to protect against the development of rabies.

Hyperimmune globulin administration is also a form of passive immunization, and it provides only temporary protection against disease development. While it is not used to
protect against an infectious disease, Rh immune globulin (RHIG) is a type of hyperimmune globulin.

**Factor VIII Concentrate**

Factor VIII is an important coagulation factor known as anti-hemophiliac factor. People who lack this factor have a coagulation disorder known as hemophilia A. Hemophilia A is an X-linked recessive disorder found in males who inherit a defective X chromosome from their mothers that does not produce (or produces insignificant amounts of) Factor VIII. Spontaneous mutations of the chromosome may also occur and a child with hemophilia A may be identified in a family with no history of the disorder.

Per the Centers for Disease Control and Prevention (CDC), hemophilia A occurs in about 1 in 5,000 live births, and about 20,000 people in the United States have hemophilia A. Hemophilia A affects all races and ethnic groups. Hemophilia A varies in severity.

Factor VIII concentrate is made two ways: derived from human plasma or produced by recombinant DNA technology. Recombinant Factor VIII is preferred for use because it is derived from hamster cell lines and it is very safe regarding the transmission of human infectious microorganisms. Factor VIII derived from pooled human plasma may be used in certain situations. In the process of purifying plasma to obtain Factor VIII, hepatitis C, hepatitis B, and HIV viruses are able to be inactivated. However, parvovirus, hepatitis A virus, and prions are still potential problems for transmission.

Factor VIII concentrate is a lyophilized powder that is reconstituted with a specific solution, and administered IV as a bolus. Some people with hemophilia A require daily or weekly infusions to prevent spontaneous bleeding; others may need less frequent administration.

**Factor IX Concentrate**

Factor IX is another important coagulation factor. People who lack Factor IX have a bleeding disorder known as hemophilia B, also known as Christmas Disease. As with hemophilia A, hemophilia B is an X-linked recessive disorder resulting in deficiency of factor IX. Spontaneous mutation can result in this disorder as well. Hemophilia B is four times less common than hemophilia A.

Preparation, administration, and frequency of dosing for Factor IX concentrate is the same as that for Factor VIII. Hemophilia B also differs in severity from person to person.

**Albumin and Protein Plasma Fraction**

Albumin and plasma protein fraction (83% albumin and 17% globulin) are also derived from pools of donor plasma that have tested negative for hepatitis C, hepatitis B, and HIV. These products are used in hypovolemic patients (those who have lost large quantities of blood) and who have low concentrations of proteins in their blood.
TRANSFUSION REACTIONS

Transfusion of blood and blood components is generally considered to be a safe procedure, and the chance of having a reaction is very small. However, reactions to transfusion do occur. Some reactions occur immediately or within 24 hours of a blood transfusion (immediate); others may not occur for weeks, months, or even years later (delayed).

The following table classifies the types of transfusion reactions as immediate or delayed.

<table>
<thead>
<tr>
<th>Immediate Transfusion Reactions</th>
<th>Delayed Transfusion Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Transfusion-associated sepsis</td>
<td>• Delayed hemolytic transfusion reaction</td>
</tr>
<tr>
<td>• Febrile (fever/chills) reaction</td>
<td>• Development of a bloodborne infection</td>
</tr>
<tr>
<td>• Hemolytic transfusion reaction</td>
<td>• Iron overload</td>
</tr>
<tr>
<td>• Allergic reactions</td>
<td>• Graft versus host disease</td>
</tr>
<tr>
<td>o Urticaria (hives)</td>
<td>• Post-transfusion purpura</td>
</tr>
<tr>
<td>o Anaphylactic reactions</td>
<td>• Platelet refractoriness</td>
</tr>
<tr>
<td>• Transfusion-related acute lung injury (TRALI)</td>
<td></td>
</tr>
<tr>
<td>• Transfusion associated circulatory overload (TACO)</td>
<td></td>
</tr>
<tr>
<td>• Reactions associated with massive transfusion</td>
<td></td>
</tr>
<tr>
<td>o Metabolic reactions</td>
<td></td>
</tr>
<tr>
<td>o Hypothermia</td>
<td></td>
</tr>
</tbody>
</table>

The most frequently seen and least severe transfusion reactions are febrile. The most serious reactions, which have a very high mortality rate, are TRALI, TACO, and acute hemolytic transfusion reactions.

A transfusion should be stopped as soon as any sign or symptom of a reaction is noticed. Unfortunately the most common early symptoms of the most serious reactions are the development of a fever and chills which are also the symptoms of a febrile reaction. However, it must be assumed that the symptoms are related to a more serious life-threatening reaction and the transfusion must be stopped. More information will follow on a transfusion reaction investigation.

ACUTE TRANSFUSION REACTIONS

Transfusion-associated Sepsis

While rare, units of red blood cells and platelets can become contaminated with a small quantity of bacteria during collection and processing. As the unit of blood or platelets is stored, the bacteria may multiply and produce endotoxins as a by-product of their growth. Refrigeration of red blood cells usually limits growth of most bacteria. However, platelet concentrates are stored at room temperature so they have a greater potential for bacterial growth and endotoxin production. The short expiration date of 5 days is set to minimize the risk of bacterial growth. Bacteria most often identified as causes of transfusion-associated sepsis include *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Acinetobacter species*, *Klebsiella species*, and *Escherichia coli*. 
When transfused, the recipient’s symptoms include fever, chills, hypotension, shock, nausea, vomiting, and respiratory distress. While all units of red blood cells and platelets are inspected prior to transfusion, it may be difficult to identify bacterial contamination by a visual inspection.

**Febrile Transfusion Reaction**

As a result of pregnancy and/or prior transfusion, people develop antibodies against antigens on white blood cells. Units of red blood cells and platelets contain some white blood cells, and when transfused, if the patient has white blood cell antibodies that correspond to antigens on the white blood cells in the donor units, a reaction may occur.

Symptoms of a febrile reaction include a temperature increase of ≥ 1°C, chills, and rigors (shaking). Sometimes recipients may experience a headache and back pain. As these are symptoms of an acute hemolytic transfusion reaction, all febrile reactions must be investigated before the transfusion can be continued.

Most febrile reactions can be successfully treated with acetaminophen. Individuals who repeatedly have febrile reactions when transfused can be pretreated with acetaminophen and diphenhydramine. Blood transfusions can also be given through filters that remove the white blood cells (leukoreduction filters). In the United States, some hospitals will only transfuse red blood cells and platelets that have already been leukoreduced at the donor center.

**Acute Hemolytic Transfusion Reaction**

An acute hemolytic transfusion reaction (AHTR) is a very serious, but rare occurrence that can result in death. It happens when the recipient’s plasma has an antibody that attacks the corresponding antigen on the transfused donor red cells. AHTR is usually the result of an ABO incompatibility but it can occur to incompatibilities in other blood group systems.

The antigen:antibody reaction results in the release of large quantities of hemoglobin in the circulation called intravascular hemolysis. Intravascular hemolysis causes acute renal failure and disseminated intravascular coagulation (DIC). The severity of AHTR depends on the amount of blood given, the rate of administration, and the recipient’s kidney, liver, and heart function. Initial symptoms are discomfort, anxiety with fever, chills, facial flushing, lower back pain, and a ‘feeling of impending doom’. The patient develops shock, low blood pressure, nausea, and vomiting. If the patient is under anesthesia and in surgery, the only symptoms may be a drop in blood pressure, uncontrolled bleeding from surgical sites, and the voiding of dark-color urine.

As soon as an AHTR is suspected, the transfusion must be stopped and supportive treatment for the patient begun. The goal of supportive treatment is to maintain the patient’s blood pressure and kidney function. The patient may need to be placed on dialysis. The degree of renal failure is related to the patient’s prognosis.

The most common cause of AHTR is clerical error, such as a mislabeled blood specimen used for pretransfusion testing or transfusion of a unit of blood meant for transfusion to another recipient. Clerical errors of these types were previously discussed.
Allergic Reactions

Urticaria

Some patients have mild allergic reactions to transfusion and develop urticaria (hives) and itching. As with most allergic reactions, this can be treated with antihistamines. Urticaria result when the patient is exposed to foreign proteins in the plasma of transfused blood and blood components. Patients with a history of mild allergic reactions can be pretreated with diphenhydramine.

Anaphylactic Reactions

Rarely transfusion recipients can develop symptoms of anaphylaxis when transfused. These symptoms include dyspnea, swelling of the larynx with resulting stridor, hypotension, tachycardia, loss of consciousness, cardiac arrhythmia, shock, and cardiac arrest.

The cause of anaphylaxis is due to the recipient having antibodies to IgA immunoglobulins. All red blood cell units and blood components contain IgA in the plasma. The recipient’s anti-IgA reacts with the donor IgA causing anaphylaxis.

People who develop antibodies to IgA immunoglobulins have a deficiency of IgA. They are born with a selective deficiency to IgA and have normal quantities of other immunoglobulins. As a result of their IgA deficiency, they develop antibodies to IgA.

Patients identified with IgA deficiencies require transfusion with red blood cells and platelets that have been ‘washed’ with saline to remove all traces of plasma. If they require FFP transfusions, the donors must be IgA-deficient.

Transfusion-Related Acute Lung Injury

Transfusion-related acute lung injury (TRALI) occurs 1-6 hours after transfusion, and it is now considered the leading cause for transfusion-related death. TRALI occurs when white blood cell antibodies in the donor plasma react with white blood cells in the recipient’s circulation. The antigen:antibody reaction activates the complement cascade, a part of the immune system that promotes an inflammatory response. As a result of the complement activation, white blood cells move into the lungs and release cytotoxic substances that react with the cells that line the lungs. The recipient develops pulmonary edema and acute respiratory distress. Mechanical ventilation of the patient may be needed until the pulmonary edema resolves.

TRALI most often occurs with the transfusion of FFP and platelets. If a patient has a history of TRALI, transfusion of FFP and platelets from men is recommended as they are less likely to have antibodies to white blood cells. Women who have been pregnant often develop white blood cell antibodies.
Transfusion-Associated Circulatory Overload

The second most common cause of transfusion-related death is transfusion-associated circulatory overload (TACO). TACO occurs when too much blood and/or blood products are transfused in a short period of time to patients, especially those patients that have pre-existing conditions such as diminished cardiac and/or renal function.

Due to the patients’ inability to handle the increased circulatory volume, they develop acute pulmonary edema. Symptoms include dyspnea, hypertension, tachycardia, and severe headache. The transfusion should be stopped and the patients should be treated for heart failure. If transfusions must be continued, smaller volumes should be given more slowly.

Reactions Associated with Massive Transfusion

Transfusion of many units of blood and blood components is often necessary to save the lives of those individuals who have been seriously injured, or who have suffered a hemorrhage as a result of a medical condition or surgical procedure. The following are generally accepted definitions of massive transfusion. Note: An average adult has a blood volume of about 10 units of blood.

- Loss of 150 mL of blood per minute
- Replacement of one entire blood volume within 24 hours
- Transfusion of >10 units of red blood cells in 24 hours
- Transfusion of >4 units of red blood cells in 1 hour when on-going need is anticipated
- Replacement of 50% of total blood volume within 3 hours

Massive transfusion can be life-saving but it can result in complications such as the following.

- Hypothermia
- Citrate toxicity
- Metabolic conditions
  - Hypocalcemia
  - Hyperkalemia
  - Hypomagnesemia
  - Acidosis
- Volume-related conditions
  - Thrombocytopenia
  - Dilution of coagulation factors

All these conditions must be monitored and treated as they arise. Hospital blood bank laboratories have massive transfusion protocols to assist with management of thrombocytopenia and dilution of coagulation factors. Recommendations generally include the transfusion of 6 units of FFP and 6-10 random donor platelets (or 1-2pheresis platelets) for every 10 units of red blood cells transfused.
Hypothermia can be prevented by using an intravenous set with a heat-exchange device that gently warms the blood as it is transfused. Laboratory monitoring of coagulation and metabolic conditions is critical to assure proper treatment is administered to the patient to manage the development of conditions related to massive transfusion.

DELAYED TRANSFUSION REACTIONS

Delayed Hemolytic Transfusion Reaction

Delayed hemolytic transfusion reactions (DHTR) commonly occur 4-8 days following a transfusion. Typically, the transfusion recipient had developed antibodies to red blood cell antigens from previous transfusions or pregnancies but, at the time of the pretransfusion testing, the antibodies were undetectable by standard testing procedures. The patient unknowingly received red blood cells that had the corresponding antigen. An antigen:antibody reaction occurred at the time of the transfusion, resulting in the patient’s antibody raising in titer (quantity). This is called an anamnestic response. The antibody then began to destroy the transfused red cells. The hemolysis that occurs is not as rapid as the hemolysis seen in an AHTR, and the patient’s life is usually not threatened.

The usual symptoms are jaundice with a decrease in hematocrit with no evidence of bleeding, and a rise in bilirubin. The reaction often is not identified until the physician orders another blood transfusion due to the patient’s drop in hemoglobin. The pretransfusion testing now easily detects the presence of the antibody when it was undetectable on the earlier testing.

Hospital transfusion departments must always keep records on patients that have unexpected antibodies as the unexpected antibodies may not always be detectable in pretransfusion testing. However, if the records indicate they were identified in the past, all future transfusions of red blood cells must be negative for those antigens to avoid the development of DHTR.

Development of Bloodborne Infections

As discussed previously, careful donor questioning, deferral (when indicated), and testing of donor units for numerous bloodborne pathogens, makes the United States blood supply safer than it has ever been. However, while rare, it is still possible for blood transfusion recipients to become infected with a bloodborne pathogen. The National Institutes of Health have the following statistics for transmission of infectious disease via blood transfusion in the United States.

- Human Immunodeficiency Virus: 1 in 2 million donor units
- Hepatitis B Virus: 1 in 270,000 donor units
- Hepatitis C virus: 1 in 2 million donor units
- West Nile Virus: 1 in 350,000 donor units
While very rare, many other infectious diseases have been reported to be transmitted through blood transmission in the United States including malaria, cytomegalovirus, Chagas disease, and syphilis.

**Iron Overload**

Many people require frequent blood transfusions as a result of chronic conditions and diseases. This includes but it not limited to individuals with sickle cell anemia, thalassemia, and kidney failure. Because excess iron cannot be excreted, these individuals have large amounts of iron built up in their liver, pancreas, endocrine glands, and heart, which can damage these organs. These individuals need to have their iron levels monitored, and when indicated, they should receive chelating agents to remove excess iron stores. If not, they can develop hepatic failure and cardiac toxicity.

**Graft Versus Host Disease**

Many patients who receive blood transfusions are immunocompromised; i.e., they do not have a functioning immune system. Often these patients have received stem cell or bone marrow transplantation and their own immune system has been killed off to allow the transplant to be successful.

Red blood cells, FFP, and platelets contain fully functioning lymphocytes, even after leukoreduction. When these products are transfused into a patient who is immunocompromised, the donor lymphocytes mount an immune response against the recipient. The recipient develops a fever, rash, vomiting, watery and bloody diarrhea, and lymphadenopathy. This is called graft versus host disease (GVHD), and it has a 90% mortality rate because there is no specific treatment available. GVHD can be prevented by irradiation of blood and blood products prior to transfusion. The irradiation kills the lymphocytes, making them unable to cause an immune response.

**Post-Transfusion Purpura**

Post-transfusion purpura (PTP) occurs about 1 week after a blood transfusion. The transfusion recipient suddenly develops thrombocytopenia with generalized purpura (small purple spots). The mechanism of PTP is not well identified. It is thought the blood transfusion stimulates the patient to develop auto-antibodies against their own platelets, thus removing them from the circulation. PTP is usually self-limiting. Patients may require therapeutic plasma exchange to remove the circulating platelet auto-antibodies.

**Platelet Refractoriness**

Hematology/oncology patients are often transfused with platelets as chemotherapeutic drugs destroy platelets. Platelet transfusions provide significant value in preventing and treating hemorrhage in this patient population. About 33% of patients fail to have a satisfactory response to platelet transfusion, leading to longer hospital stays and increased morbidity and mortality. These patients are said to have platelet refractoriness.
The most common cause of platelet refractoriness is the development of antibodies to antigens (HLA and PLA system antigens) on the transfused platelets. Patients make these antibodies as a result of prior transfusions, pregnancies, organ transplantations, and bone marrow/stem cell transplantations. In cases of platelet refractoriness, it is necessary to transfuse platelets that are HLA and PLA antigen compatible with the HLA and PLA antibodies in the recipient.

**SUSPECTED TRANSFUSION REACTION INVESTIGATION**

When any of the following signs or symptoms are observed, a transfusion should be stopped.

- Inflammatory reactions: fevers, chills, skin changes, pain at the infusion site
- Circulatory changes: blood pressure changes, shock, hemoglobinuria
- Pulmonary symptoms: dyspnea, orthopnea, wheezing, respiratory failure
- Coagulation symptoms: unexplained increase in bleeding, DIC
- Psychological changes: sudden anxiety, sense of unease, feeling of ‘impending doom’

Until proven otherwise, all transfusion reactions should be assumed to be hemolytic. After the transfusion is stopped, a clerical check should be performed. This includes the following.

- Check the transfusion paperwork with the unit identification and the patient identification to assure the right unit went to the right patient.
- Notify the Blood Bank personnel of the transfusion reaction so they can perform a clerical check on the specimen used to perform the testing and the unit of blood released for the transfusion.
- Collect a post-transfusion EDTA specimen for a laboratory investigation of a suspected transfusion reaction.
  - The specimen should be centrifuged to observe the plasma for the presence of hemolysis. The presence of hemolysis in a non-traumatic venipuncture could indicate a hemolytic transfusion reaction has occurred.
  - A DAT (direct antiglobulin test) should be performed. A positive DAT may indicate a hemolytic transfusion reaction has occurred.
  - The ABO and Rh typing should be performed on the post-transfusion reaction specimen. In the event of an ABO hemolytic transfusion reaction, a type of agglutination called ‘mixed field’ will be observed due to the presence of two ABO types in the patient’s bloodstream.

If a hemolytic transfusion reaction has occurred, the patient will experience issues with inflammation, coagulation, circulation, and renal function. Management of the patient’s blood pressure and kidney function are of the utmost importance.

If there is no evidence that hemolytic transfusion reaction has occurred, testing for the other types of transfusion reactions can begin. This testing can include more tests in the blood bank department, and other tests in the chemistry, hematology, and
microbiology departments of the laboratory. Radiology studies are often necessary to identify issues such as TRALI and TACO.

HEMOLYTIC DISEASE OF THE NEWBORN

Hemolytic disease of the newborn (HDN) begins in utero. Historically, the disease was called hydrops fetalis and babies were born with total body swelling, breathing problems, bruising or purplish spots on the skin, heart failure, severe anemia, and severe jaundice. Some babies were stillborn, others died shortly after birth.

Rh HEMOLYTIC DISEASE OF NEWBORN

In 1932, the hydrops fetalis disease was renamed erythroblastosis fetalis as it was discovered that babies born with these symptoms had erythroblasts, immature red blood cells, in their circulation. In 1953, the pathogenesis of this disease was identified, and it was renamed Rh hemolytic disease of the newborn or hemolytic disease of the fetus and newborn (HDFN). The pathogenesis of the disease follows.

1st Pregnancy

- Rh negative woman carries Rh positive fetus.
- During pregnancy, a small quantity of fetal Rh positive blood cells may enter mother’s circulation; this is a normal process. The mother may begin to develop anti-D antibodies. However, the first antibodies produced are IgM and this antibody type does not cross into the placenta.
- At birth, fetal blood cells enter the maternal circulation. Most Rh negative women will develop anti-D antibodies at this time.

2nd pregnancy

- If the mother carries an Rh positive fetus, during the gestation her anti-D antibodies will cross into the placenta. The mother now has IgG anti-D and this immunoglobulin type easily crosses through the placenta.
- The maternal anti-D attacks the fetal red blood cells with the D antigen.
- Fetus becomes anemic due to the antigen:antibody reactions.
- Liver and spleen increase in size as they try to produce more red blood cells to compensate for the anemia.
- If hemolysis is mild or moderate:
  - It can be tolerated by the fetus.
  - At birth, the baby may require no treatment, or may require transfusion for anemia, and treatment for jaundice.
- If the hemolysis is severe:
  - Intrauterine transfusions may be needed to treat the severe anemia of the fetus.
  - Fetal tissues become swollen due to the accumulation of excess fluids around organs, including the heart. This can lead to fetal death, or death shortly after birth.
  - At birth, the baby’s bilirubin may rise to dangerous levels within 24 hours causing kernicterus, a potentially fatal brain condition that leaves permanent neurological damage in babies that survive.
Subsequent pregnancies

- With each subsequent pregnancy with an Rh positive fetus, the Rh negative mother is exposed to more D positive red cells. This increases the titer (amount) of the anti-D antibody.
- The higher the titer of antibody, the more severe the disease will be in an Rh positive fetus.
- Historically, Rh negative mothers with high titers of anti-D are unable to deliver live Rh positive babies.

Since the administration of Rh immune globulin in all Rh negative women antepartum, it’s readministration to women who deliver Rh positive babies, and in all other pertinent circumstances (such as abortions, miscarriages, amniocentesis, etc. where it is not possible to determine the Rh type), Rh hemolytic disease of the newborn is becoming a rare occurrence.

HEMOLYTIC DISEASE OF THE NEWBORN DUE TO OTHER BLOOD GROUP SYSTEMS

Maternal antibodies to antigens in other blood group systems can cause HDN, but the disease produced by the other antibodies is not as serious as the disease caused by anti-D. It is not unusual for group A (or more rarely, group B) babies born of group O mothers to have mild HDN caused by maternal anti-A or anti-B in the form of anti- A,B. As previously mentioned, anti-A,B is an IgG antibody; anti-A is IgM. IgM antibodies are too large to cross into the placenta; however, anti-A,B can cross into the placenta and cause mild destruction of group A or group B red cells.

Fortunately, these other antibodies do not cause a disease as serious as the disease seen with anti-D. Monitoring and treatment of jaundice after birth is usually sufficient to assure a positive outcome for the baby.
REGULATORY AND ACCREDITING AGENCIES

All practices related to donor blood collection and transfusion of blood and blood components are highly regulated to assure the safety of both the blood donor and the transfusion recipient.

The Food and Drug Administration (FDA) is mandated by federal law to oversee all practices related to blood donor collection and transfusion. Transfusion of blood and blood components is considered a drug treatment, and it therefore falls under the authority of the FDA. The FDA is mostly concerned with the inspection of donor centers and aspects of blood collection, manufacturing of components, and distribution of blood and blood products to hospital transfusion services. However, they have the right to inspect any hospital transfusion service at any time, primarily following the reporting of an adverse event. FDA inspections are unannounced, and all donor centers and hospital transfusion services are inspected at least every two years.

The following organizations and agencies are known as ‘Deemed Status Accrediting Organizations’ which means they can determine that a laboratory meets the Clinical Laboratory Improvement Amendment (CLIA) requirements as required by the Centers for Medicare and Medicaid Services (CMS). These organization’s criteria must meet, but may actually exceed CLIA requirements. Many hospital transfusion services and donor centers are voluntarily inspected by all three of the following organizations.

- AABB (formerly known as the American Association of Blood Banks): Donor centers and transfusion services laboratories voluntarily participate in AABB’s accreditation program to optimize patient and donor care and safety.
- College of American Pathologists (CAP): CAP voluntarily inspects and accredits hospital laboratories, including the transfusion service department. If the transfusion service is accredited by AABB, the inspection is generally minimal.
- The Joint Commission (TJC): TJC voluntarily accredits and certifies healthcare organizations in the United States. Transfusion service departments within the laboratory are part of the inspection and accreditation process. However, if a transfusion service is accredited by AABB or CAP, TJC will accept that accreditation and often just do a ‘walk through’ in lieu of an entire inspection.

CONCLUSION

This CE course reviewed the key concepts of blood donation, transfusion, adverse effects of transfusion, and hemolytic disease of the newborn. The ABO and Rh blood group systems discovered in the 20th century remain the most important blood group systems in blood transfusion in the 21st century.

Transfusion of blood and blood components can be life-saving. But failure to properly identify a patient before collecting a specimen, to place a correct label on a tube of blood, or to check patient identification before beginning a transfusion can cause a fatal hemolytic transfusion reaction. In the healthcare environment, there is zero tolerance for errors in the positive identification of patients for transfusion. Careful attention to detail and avoiding distraction is important during all specimen collection and transfusion administration procedures.
REFERENCES


QUESTIONS
An Introduction to Transfusion Medicine #1220319

Directions:
• Answer sheets: Read the instructions to assure you correctly complete the answer sheets.
• Online: Log in to your User Account on the NCCT website www.ncctinc.com.
  o NOTE: If the online test questions differ from the course test that follows the reading material, the CE course you are using is outdated or the question has
1. About how many units of red blood cells are needed daily in the United States?
   a. 21 million
   b. 36,000
   c. 10,000
   d. 7,000

2. A hospital blood bank laboratory is more appropriately called __________.
   a. Donor Center
   b. Hematology
   c. Immunology
   d. Transfusion Services

3. Antigens are __________, and are found primarily __________.
   a. Immunoglobulins; in the plasma
   b. immunoglobulins; on the surfaces of cells
   c. proteins and polysaccharides; in the plasma
   d. proteins and polysaccharides; on the surfaces of cells

4. Antibodies are __________, and are found primarily __________.
   a. immunoglobulins; in the plasma
   b. immunoglobulins; on the surfaces of cells
   c. proteins and polysaccharides; in the plasma
   d. proteins and polysaccharides; on the surfaces of cells

5. A term that is interchangeable with ABO blood type is __________.
   a. Class
   b. System
   c. Rh type
   d. Group

6. A mother that is group AB and a father that is type O can have children of which of the following ABO blood types?
   a. A, B
   b. A, B, and O
   c. A, B, and AB
   d. A, B, O, and AB
7. If a mother and father are both type B, they can have a type O baby:
   a. True
   b. False

8. If both parents are type O, their children could be which of the following blood types?
   a. A, B
   b. A, B, O
   c. O
   d. A, B, AB, or O

9. A group B individual has which of the following antibodies in his/her plasma?
   a. Anti-A
   b. Anti-B
   c. Anti-A and anti-A,B
   d. Anti-B and anti-A,B

10. What makes transfusion of compatible ABO red blood cells of the utmost importance?
    a. The strong naturally-occurring anti-A and anti-B antibodies in the donor plasma
    b. The strong naturally-occurring anti-A and anti-B antibodies in recipient plasma
    c. The strong naturally-occurring A and B antigens on the donor cells
    d. The strong naturally-occurring A and B antigens on the recipient cells

11. What is the recommended evacuated tube for ABO typing?
    a. EDTA (lavender or pink closure)
    b. Heparin (green closure)
    c. No additive (red/gold closure)
    d. Sodium citrate (light blue closure)

12. Following are the results of an ABO typing. What is the ABO blood type of this individual?

<table>
<thead>
<tr>
<th>Forward Typing</th>
<th>Reverse Typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
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</tbody>
</table>

   a. A
   b. B
   c. AB
   d. O
13. Referring to the table on page 14, what percentage of the Hispanic population in the United States is O Positive?

   a. 37%
   b. 25%
   c. 53%
   d. 1%

14. In addition to the ABO and Rh blood group systems, how many other blood group systems are there?

   a. 300
   b. 33
   c. 12
   d. 10

15. An antibody screen test is also known as a/an __________.

   a. Antibody identification panel
   b. Crossmatch
   c. Direct antiglobulin test
   d. Indirect antiglobulin test

16. An antibody screen test is used to determine which of the following?

   a. ABO type
   b. Rh positive or negative
   c. The presence of unexpected antibodies
   d. The specific identity of an unexpected antibody

17. When is an antibody identification panel needed?

   a. When an ABO incompatible transfusion has occurred
   b. When an antibody screen test was positive
   c. Prior to all transfusions
   d. To screen for the presence of unexpected antibodies

18. What is the most recent FDA guidance on blood donor eligibility for men who have sex with men?

   a. Temporarily deferred for 12 months after the most recent sexual contact with another man
   b. Temporarily deferred for 24 months after the most recent sexual contact with another man
   c. Temporarily deferred for 20 years after the most recent sexual encounter with another man
   d. Indefinite deferral
19. Donor blood in the United States is tested for all of the following bloodborne pathogens except __________.
   a. Human Immunodeficiency Virus, Type I
   b. malaria
   c. syphilis
   d. hepatitis C

20. What is the earliest a pre-transfusion blood specimen can be drawn from an intended recipient of an upcoming transfusion?
   a. If a blood type history is already on record, no pre-transfusion testing is needed
   b. The day of the transfusion
   c. Up to 2 days prior
   d. Up to 3 days prior

21. Which of the following pieces of information is required to be included on the label of specimens collected for blood bank testing that is usually not required for other laboratory department testing?
   a. Date
   b. Identification of the person collecting the specimen
   c. Patient first and last name
   d. Patient unique identification number

22. Barcode identification systems and use of a second patient identification band system are utilized for which of the following purposes?
   a. To assure compatible blood is transfused
   b. To eliminate clerical error 100%
   c. To assure safer transfusions
   d. To minimize transfusion reactions

23. Misidentification of patients and mislabeling of tubes of blood by individuals who collect blood specimens can result in a fatal blood transfusion reaction.
   a. True
   b. False

24. Fresh frozen plasma is transfused to patients who have lost large quantities of blood as it supplies __________.
   a. a temporary source of coagulation factors
   b. white blood cells needed to fight infection
   c. platelets needed to help the blood clot
   d. a fibrin glue to reduce bleeding
25. Which of the following blood components is often transfused to patients receiving chemotherapy?
   a. Cryoprecipitate
   b. Fresh frozen plasma
   c. Platelets
   d. Rh immune globulin

26. Which of the following products is given to an individual who has hemophilia A?
   a. Cryoprecipitate
   b. Factor VIII concentrate
   c. Factor IX concentrate
   d. FFP

27. Which of the following is the most frequently seen transfusion reaction?
   a. Allergic – urticaria
   b. Febrile
   c. Hemolytic transfusion reaction – acute
   d. Transfusion-associated acute lung injury

28. Which practice can help reduce the number of febrile transfusion reactions?
   a. Leukoreduction of the blood products
   b. Irradiation of the blood products
   c. Bloodborne pathogen testing of the blood products
   d. Treatment with antihistamines

29. What is the most common cause of an acute hemolytic transfusion reaction?
   a. Analytic error
   b. Clerical error
   c. Donor center error
   d. Physician error

30. Which of the following is considered to be the leading cause of transfusion related death?
   a. Acute hemolytic transfusion reactions
   b. Anaphylactic reactions
   c. TACO
   d. TRALI
31. Based on statistics from the National Institutes of Health, which of the following infectious diseases are most likely to result from a blood transfusion in the United States?

   a. Cytomegalovirus  
   b. HIV  
   c. Hepatitis B  
   d. Hepatitis C

32. What product is given to Rh Negative women when they deliver an Rh Positive baby or have a circumstance where the Rh type of the fetus cannot be determined?

   a. Hyperimmune rubella globulin  
   b. Protein plasma fraction  
   c. Rh immune globulin  
   d. RSV immune globulin

*end of test*
**P.A.C.E.® Program Evaluation**

Directions: Please let us know whether this CE course met your expectations by answering the following questions. Your feedback helps us to make our products better for you!

<table>
<thead>
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<th>Course #: 1220319</th>
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**OBJECTIVES**

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<tr>
<th>____ Yes</th>
<th>____ No</th>
<th>1. Did you meet the objectives while reading this CE course?</th>
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<tr>
<td>____ Yes</td>
<td>____ No</td>
<td>2. Did the test measure what you learned?</td>
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**COURSE CONTENT**

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<th>3. Were you satisfied with this course?</th>
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<td>4. Was the CE course organized and useful for learning?</td>
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<td>____ Yes</td>
<td>____ No</td>
<td>5. Was this CE course written at the right level for the practicing professional?</td>
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**VALUE**

<table>
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<tr>
<th>____ Yes</th>
<th>____ No</th>
<th>6. Did you learn anything new?</th>
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<tr>
<td>____ Yes</td>
<td>____ No</td>
<td>____ Maybe 7. Did you learn anything you might use at work?</td>
</tr>
</tbody>
</table>

What can NCCT do to make the CE courses better for you?

What would you like to learn about in the future? Please list specific topics!

*Please include this evaluation with your answer sheet.*