Edvo-Kit # 142

Edvo-Kit #142

The Science Behind Finding a Perfect Match: Blood Typing for Stem Cell Transplants

Experiment Objective:

In this experiment, students will take on the role of transplant physicians. They will analyze simulated blood samples for transplant compatibility and compare genetic markers to identify which donor is the best match for a leukemia patient in need of a stem cell transplant.

See page 3 for storage instructions.

Version 142.250711

SAMPLE LITERATURE
Please refer to included weblink for correct version

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Experiment Components

	Component	Storage	Check ✓
A	Simulated white blood cell counting sample	Room Temp.	❑
B	Control Simulated Blood Sample Type A	Room Temp.	❑
C	Control Simulated Blood Sample Type B	Room Temp.	❑
D	Control Simulated Blood Sample Type AB	Room Temp.	❑
E	Control Simulated Blood Sample Type O*	Room Temp.	❑
F	Anti-A Serum	Room Temp.	❑
G	Anti-B Serum	Room Temp.	❑
H	Anti-Rh Serum	Room Temp.	❑
I	AHG Serum	Room Temp.	❑
J	Red Dye Concentrate	Room Temp.	❑

This experiment is designed for 10 groups.

NOTE: All blood samples will be prepared by instructor just prior to use.

**Empty bottle to be filled by instructor.*

Supplies *(included with this experiment)*

	Check ✓
• Transfer pipets	❑
• Microtiter plates	❑
• Microcentrifuge tubes	❑
• Counting chambers	❑

Requirements *(NOT included with this experiment)*

- Automatic micropipettes (5 – 50 µL) and tips
- Microscopes
- Distilled or deionized water
- Bleach solution or lab disinfectant

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals. No actual blood or blood products are used in this experiment. None of the experiment components are derived from human sources.

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Background Information

CANCER AND LEUKEMIA

Cancer is a major health issue that affects millions of people around the world. As of 2020, cancer accounted for approximately 1 out of every 6 deaths worldwide. Cancer is a disease that starts when cells in the body begin to grow out of control. Normally, our cells grow, divide, and die in a very controlled way but in cancer, something goes wrong with this process. The cells keep growing when they shouldn't, forming lumps called tumors or spreading through the body. There are many types of cancer, and they can affect almost any part of the body. Learning about cancer helps us understand how the body works, how diseases develop, and how scientists and doctors work to find treatments and cures.

Leukemia is a type of cancer that starts in the blood and bone marrow, where blood cells are made. Unlike other cancers that form solid tumors, leukemia causes the body to make large numbers of abnormal white blood cells, which don't work properly. These cells can crowd out healthy ones, making it hard for the body to fight infections, carry oxygen, and stop bleeding. In this experiment, we'll take a closer look at what leukemia is, how it affects the body, and how doctors diagnose and treat it.

Leukemia is classified in two main ways:

Acute vs. Chronic

- ***Acute leukemia*** progresses quickly and requires immediate treatment.
- ***Chronic leukemia*** develops more slowly and may go unnoticed for years.

Myeloid vs. Lymphoid

- ***Myeloid leukemia*** affects the myeloid cell line, which gives rise to red cells, platelets, and some types of white cells.
- ***Lymphoid leukemia*** affects lymphocytes, a type of white blood cell involved in the immune response.

Treatment for leukemia depends on the type and how fast it develops. Common treatment options include chemotherapy (drugs that kill cancer cells), radiation therapy (high doses of radiation that damage DNA of cancer cells), and targeted therapy (drugs that attack specific parts of cancer cells). In recent years, new treatments like immunotherapy—which helps the body's immune system fight cancer—have shown promising results.

One of the most powerful treatments for certain types of leukemia is a stem cell transplant. This procedure replaces the damaged bone marrow with healthy stem cells—immature cells that can grow into all types of blood cells. These stem cells can come from the patient (autologous) or from a donor (allogenic). Before the transplant, patients often receive high doses of chemotherapy or radiation to destroy as many leukemia cells as possible. Next, donor stem cells are infused into the patient's blood stream where they will travel to the bone marrow and grow and produce new, non-cancerous blood cells. Stem cell transplants can offer a chance for long-term remission or even a cure, especially when other treatments haven't worked. In this unit, we'll explore how leukemia affects the body and how stem cell transplants are used to help patients rebuild a healthy blood system. During allogeneic stem cell transplants, healthy stem cells are taken from a closely matched related or unrelated donor. When someone volunteers to be a stem cell donor, their blood is extracted and carefully screened and tested to determine if they will be a good match for the patient.

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White blood cells (leukocytes) are immune cells that defend the body against infections, cancer, and foreign invaders. A white blood cell (WBC) count measures the number of white blood cells in a given volume of blood, typically reported as: WBC count = cells per microliter (µL) of blood. While not the only diagnostic criteria, a high white blood cell count, known as leukocytosis, can be an indication of leukemia (Figure 1). The rise is often due to the uncontrolled production of immature or abnormal white cells (blasts). These blasts do not function normally, and they crowd out healthy blood cells. A WBC count of 50,000–300,000/µL or more can be seen in leukemia, especially chronic myeloid leukemia or untreated acute leukemia.

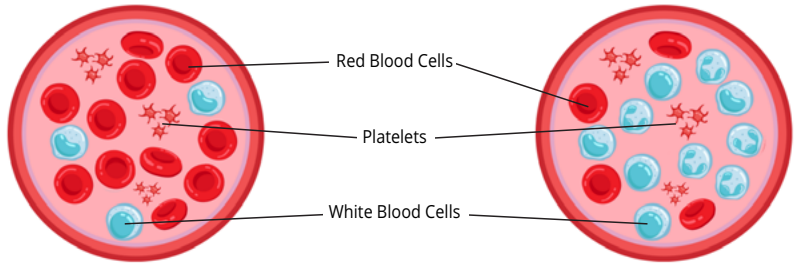

Figure 1: Blood from a healthy person vs. someone with leukemia.

Performing a manual white blood cell count is one of two ways to count WBCs. In manual WBC counting, which is used in some specialized or resource-limited settings, a drop of blood is diluted with a special fluid that stains the WBCs while lysing red cells. The diluted sample is then placed into a hemocytometer, a precision glass slide with a grid etched into it. Under a microscope, a technician counts the number of WBCs in designated squares of the grid and calculates the concentration of cells per microliter based on the dilution factor. The second way is to do an automated white blood cell count which uses an automated hematology instrument to count WBCs.

BLOOD TYPING

During a donor blood screening the blood undergoes ABO and Rh typing. The purpose of this test is to identify a person's basic blood group and Rh status. There are four different blood types: A, B, AB, and O and two possible Rh statuses: positive and negative. The type of blood is determined by the type of antigen the blood cells carry on the outer membrane. For example, individuals with type A antigens will have type A blood, while those with type B antigens will have type B blood. Blood cells carrying both type A and type B antigens are considered type AB blood and blood with neither antigen is type O blood. In addition to the ABO system, the Rh system further classifies blood as either positive or negative based on the presence or absence of the Rh antigen (also called the D antigen). The Rh factor is a protein that is found on the surface of red blood cells. If a person is Rh positive, this means that they have those Rh factors present in their blood. Importantly, each person produces antibodies that are directed against the A or B antigens that are not present on their red blood cells. For example, someone with type A+ blood has both A antigens and the Rh antigen. They will produce B antibodies but no D antibodies. In contrast, someone with type A- blood has A antigens but no D antigen, and will produce Anti-B antibodies in the plasma. Because of this, a person can only receive blood if there are matching antigens present. AB+ blood type is known as the universal receiver. They can receive A,B, AB,

Blood Type	Antigens on RBSs	Antibodies in Plasma	Can Receive From
A	A	anti-B	A,O
B	B	anti-A	B,O
AB	A and B	none	A,B,AB, O
O	none	anti-A, anti-B	O only

Rh Factor Compatibility:

- Rh+ (has the D antigen) ➔ Can receive Rh+ or Rh-
- Rh- (no D antigen) ➔ Can receive only Rh-

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or O type blood since they have both A and B antigens and D antigens. O- blood is considered the universal donor because there are no antigens present; however this means that they can only receive type O- blood.

The process of blood typing is performed by mixing a sample of blood Anti-A serum, Anti-B serum, and Anti-D (Rh) serum respectively. If the antigen is present, the corresponding antibodies targeting that antigen will bind on the cells and agglutinate or clump together. This is critically important in transplants and transfusions. If a person with type A negative blood receives a transfusion of type B positive blood, their immune system will recognize both the B antigens and the Rh antigen as foreign. The anti-B antibodies already present in their plasma will target the B antigens, and if the person has been sensitized to the Rh antigen—either through previous transfusions or pregnancy—they may also produce anti-Rh antibodies. These antibodies bind to the foreign antigens on the transfused red blood cells, causing them to agglutinate.

CROSSMATCHING

Even if the blood type is an exact match, it does not necessarily mean that the donor's blood will be compatible with the recipients. This is because the body's immune system may detect other antigens on the donor's blood cells as foreign and attack or reject them. A blood crossmatch must be performed in order to test whether the recipient's immune system will attack the donor cells. A crossmatch is important because it helps to prevent a transfusion reaction which can be life-threatening. A cross match combines a sample of the patient's blood with a sample of the donor's blood. The samples are then incubated, centrifuged, and are combined with a testing serum such as anti-human globulin. A positive crossmatch is indicated by either agglutination or hemolysis. A positive result means that the blood is incompatible and that the recipient's antibodies will attack the donor cells. A negative crossmatch will have no hemolysis or agglutination and indicates that it is safe to proceed with the donor blood (Figure 2).

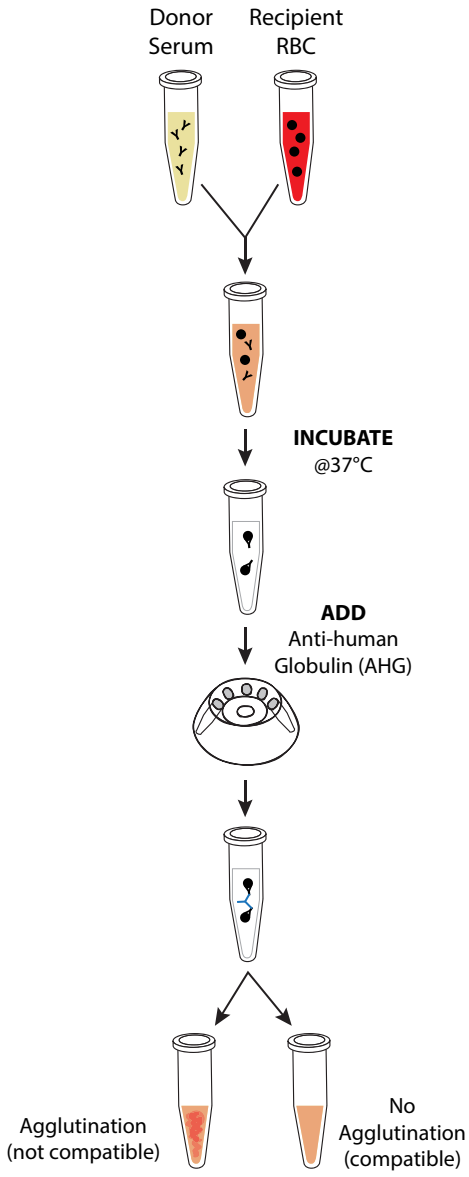

Figure 2: Process of blood crossmatching.

HLA TYPING

Another key aspect of organ and stem cell transplants is human leukocyte antigen typing (HLA typing). Human Leukocyte Antigen is a group of genes on chromosome 6 that encode proteins expressed on the surface of almost all cells. These proteins are part of the Major Histocompatibility Complex (MHC) in humans. They play a critical role in the immune system by presenting protein fragments (antigens) to immune cells to help distinguish self from non-self. There are two classes of HLA molecules. Class I molecules include HLA-A, HLA-B, and HLA-C. These present peptides to CD8+ cytotoxic T cells and are expressed on almost all nucleated cells. Class II molecules include HLA-DR, HLA-DQ, and HLA-DP. These present peptides to CD4+ helper T cells and are mainly expressed on antigen-presenting cells (dendritic cells, macrophages, B cells). HLA tests often compare the alleles at the

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HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci, but can also include HLA-C or HLA-DQ. Each person typically has two alleles at each of the six key HLA loci used in matching. A perfect match (10/10, 8/8, or 12/12 depending on how many alleles are being compared) means all alleles match between donor and recipient. Humans inherit one HLA haplotype from each parent, so parents and children are automatically haploidentical to each other (Figure 3). A haploidentical match means that the donor and recipient share exactly half (50%) of their HLA genes. It's often referred to as a "half-match." A high HLA match is important because it lessens the risk of dangerous or fatal post transplant complications such as graft-versus-host disease and graft rejection.

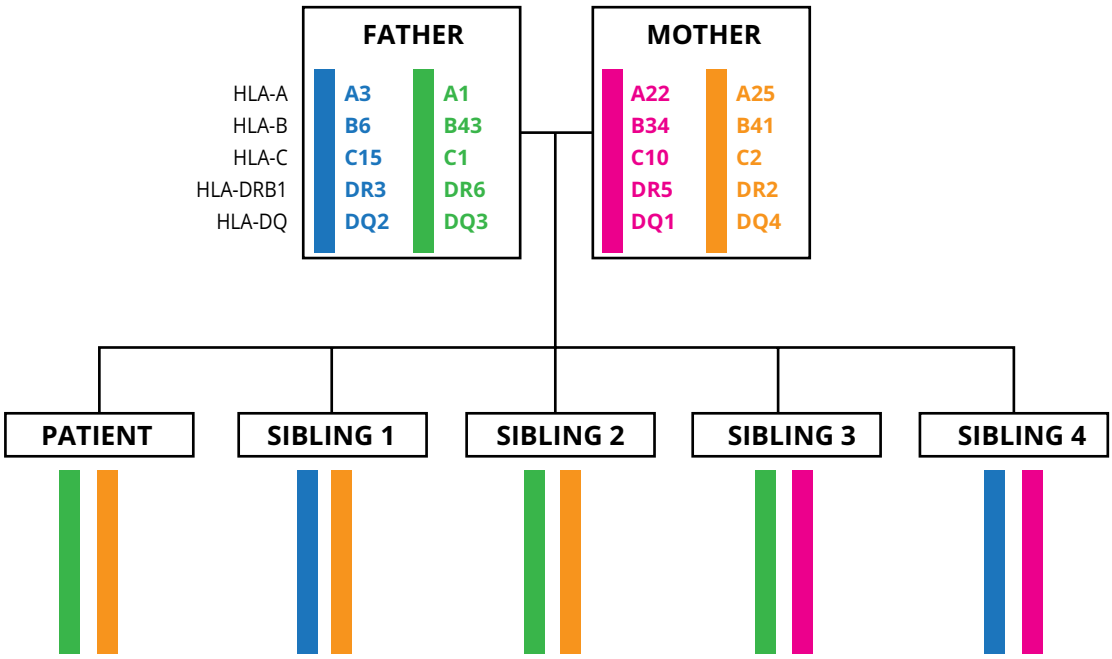

Figure 3: Example of HLA inheritance.

THE SCENARIO:

Patient presents to the Emergency Room with complaints of persistent fatigue, unexplained weight loss, recurrent infections, easy bruising and bleeding, and bone pain. She has a known history of leukemia, which had been in remission for the past year. Given her current symptoms, there is concern for a possible relapse. Initial blood tests revealed a markedly elevated white blood cell count. A subsequent bone marrow biopsy confirmed a relapse of leukemia. Due to the limited effectiveness of her previous chemotherapy regimen, the oncology team has recommended proceeding with a stem cell transplant. Potential donors from the Be The Match registry (Good Samaritan donors) are currently being evaluated. In this experiment, you will use manual white blood cell counting to obtain a pre-transplant initial white blood cell count. You will then perform different tests to determine which donor will be the best match for a therapeutic stem cell transplant.

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Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will take on the role of transplant physicians. They will analyze simulated blood samples for transplant compatibility and compare genetic markers to identify which donor is the best match for a leukemia patient in need of a stem cell transplant.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:
- Record (draw) your observations, or photograph the results.
- Record any challenges faced while performing the experiment.

After the Experiment:
- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

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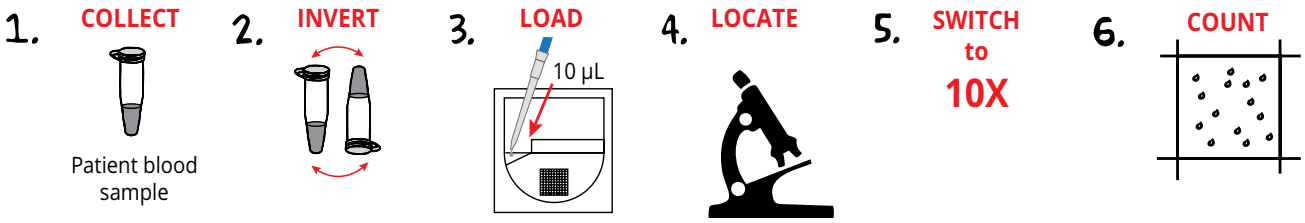
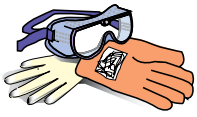
Module I: White Blood Cell Count



1. **OBTAIN** the simulated patient blood sample
2. **INVERT** for several seconds to make sure the white blood cells are suspended
3. **LOAD** 10 µL of the Patient Blood Sample into the hemocytometer
4. Using a low magnification, **LOCATE** the hemocytometer grid under the microscope
5. Once the grid is located, **SWITCH** the magnification to the 10x objective
6. **COUNT** the number of cells in one of the white blood cell counting grids. **COUNT** the number of cells in all nine squares of the hemocytometer. ***NOTE: count all any cells the top and left borders of each square, but not those that touch the bottom and right borders***
7. **RECORD** the number of cells in the Data Analysis Table Provided
8. **CALCULATE** the average number of cells per grid by dividing the total number of cells by 9. Use the formula provided to calculate the number of cells per microliter of blood. A WBC of 50,000-300,000 is an indicator of leukemia. ***NOTE: the blood sample used in this activity have been diluted by a factor of 100***



Wear gloves and safety goggles

WBC/µL= (average number of cells per grid) x (dilution factor) / volume of sample per grid square (0.1 µL)

Grid	1	2	3	4	5	6	7	8	9
Cells									

Average:_____

Number of white blood cells per µL: _____

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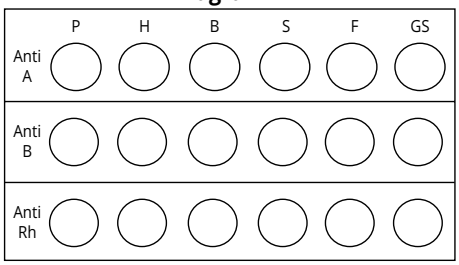
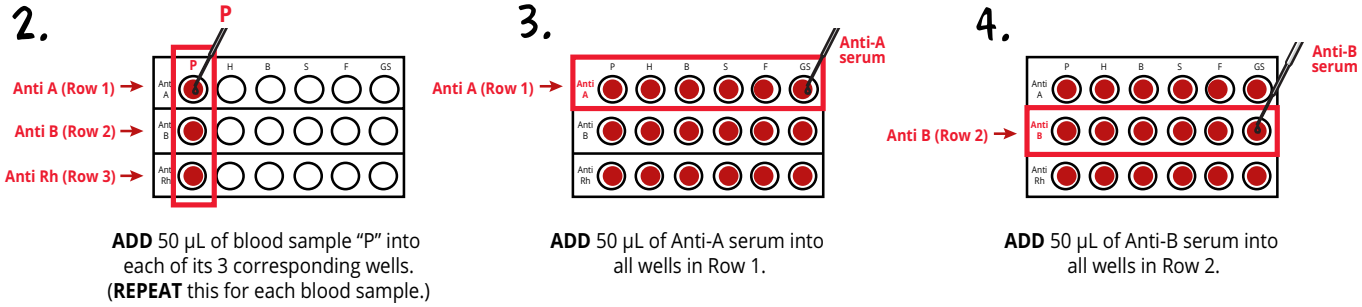
Module II: ABO Rh Blood Typing

1. **PLACE** a 6 x 3 microtiter plate piece as shown in Diagram 1. Using a laboratory marking pen, **LABEL** the 6 columns from left to right, as: P, H, B, S, F, and GS. These letters correspond with the donor blood samples: P=patient, H=husband, B=brother, S=sister, F=friend, and GS=good samaritan.

   Next, on the left side of the plate, **LABEL** Row 1 as "Anti-A", Row 2 as "Anti-B", and Row 3 as "Anti-Rh". The plate should look as shown in Diagram 1.

Diagram 1





ADD 50 µL of blood sample "P" into each of its 3 corresponding wells. (**REPEAT** this for each blood sample.)

ADD 50 µL of Anti-A serum into all wells in Row 1.

ADD 50 µL of Anti-B serum into all wells in Row 2.

ADD 50 µL of Anti-Rh serum into all wells in Row 3.

INCUBATE for 10 min.

2. **ADD** 50 µL (or two drops from a transfer pipet) of blood sample "P" into each of the its three corresponding wells under the letter "P". **REPEAT** the same procedure for each of the blood samples, using a new pipette tip for each sample. Each well requires 50 µL of blood sample.
3. Use a new pipette tip to **ADD** 50 µL of Anti-A serum into each of the wells in Row #1 (Anti-A). The same tip or transfer pipet can be used for all samples in Row #1.
4. Use a new pipette tip to **ADD** 50 µL of Anti-B serum into each of the wells in Row #2 (Anti-B). The same tip or transfer pipet can be used for all samples in Row #2.
5. Use a new pipette tip to **ADD** 50 µL of Anti-Rh serum into each of the wells in Row #3 (Anti-Rh). The same tip or transfer pipet can be used for all samples in Row #3
6. Let the samples **INCUBATE** undisturbed on the lab bench for 10 minutes.
7. **OBSERVE** the wells for the presence or absence of agglutination. Agglutination has occurred if the mixture appears to be granular and thick rather than smooth and watery. **RECORD** your results in Diagram 1 and in the chart below. **SAVE** samples for Module IV.

Sample	Patient (P)	Husband (H)	Brother (B)	Sister (S)	Friend (F)	Good Samaritan (GS)
Blood Type	AB+					

NOTE: The patient blood type has been filled in for you as this will serve as your control.

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Module III: HLA Typing

In this activity, you will analyze the patient's HLA type and compare it to the possible donors. When looking for an HLA match, doctors strive for at least a 4/8 match. You will be provided with a reference table containing genomic sequences of different HLA alleles. Using the reference table: 1) Identify the specific HLS genes present in each donor. 2) Compare each donor's alleles to the patient's to determine how many matches there are at each HLA locus. 3) Record your findings in the chart on page 13. One has been done for you as an example. Once complete, proceed to **Module IV** with each donor blood sample that is a **4/8 or higher** match to the patient sample.

REFERENCE TABLE	
Allele	**Sequence Fragment**
A*02:01	ACGGAGCTCGTGGAGACCAGGCCTGCAGGGGA
A*24:02	ATGGCCGTCATGGCGCCCCGAACCCTCGTCCTG
A*11:01	ATGGTGCTGCGGTGCTGCGCAGGGGCCCGAGC
A*26:254	ATGGTGCTGCGGTGCTGCGTAGGGGCCCGGAC
A*01:01	TCCCATTGGGTGTCGGGTTTCCAGAGAAGCCA
A*03:01	CTCCCCAGACGCCGAGGATGGCCGTCATGGCG
Allele	**Sequence Fragment**
B*07:527	CACCCACCCGGACTCAGAGTCTCCTCAGACGC
B*08:01	GATCAGGACGAAGTCCCAGGTCCCGGACGGGG
B*35:01	CACCCACCCGGACTCAGAATCTCCTCAGACGCC
B*44:02	AACCTATGTCGGGTCCTTCTTCCAGGATACTCGTG
B*15:01	ATGCGGGTCACGGCGCCCCGAACCGTCCTCCT
Allele	**Sequence Fragment**
C*07:02	AAAGGGTGGGAGGCAGGGAGTCCAGTTCAGGGA
C*04:01	CGCAGCCTGGGGGTCTCTCCCTGGTTTCCACAG
C*03:03	GGCGCAGCCTGGGGGTCTCTCCCTGGTTTCCAC
C*12:143	GGGAGGGAAACGGCCTCTGCGGAGAGGAGCGA
C*06:02	TGTTTAAAGGTTTGATTCCAGCTTTTCTGAGTCCTT
Allele	**Sequence Fragment**
DRB1*03:19	TTATTATGAATCTCTTTTAACCTTTCTATACTTG
DRB1*14:84	TATTTTTCCCCAGCTATGTTGTTATCATTTCCA
DRB1*04:90	TCAATTATTAAAATTAATTCTAGCTACTCTGTGG
DRB1*08:32	GGTGAGAGCTTCACAGTGCAGCGGCGAGTCC
DRB1*14:28	ATGGTGTGTCTGAGGCTCCCTGGAGGCTCCTG

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Module III: HLA Typing, continued

Recipient			
Patient	**Locus**	**Sequence 1**	**Sequence 2**
	A	ACGGAGCTCGTGGAGACCAGGCCTGCAGGGGA	ATGGTGCTGCGGTGCTGCGCAGGGGCCCGAGC
	B	AACCTATGTCGGGTCCTTCTTCCAGGATACTCGTG	CACCCACCCGGACTCAGAGTCTCCTCAGACGC
	C	GGGAGGGAAACGGCCTCTGCGGAGAGGAGCGA	TGTTTAAAGGTTTGATTCCAGCTTTTCTGAGTCCTT
	DRB1	TATTTTTCCCCAGCTATGTTGTTATCATTTCCA	GGTGAGAGCTTCACAGTGCAGCGGCGAGTCC

Donors			
Brother	**Locus**	**Sequence 1**	**Sequence 2**
	A	TCCCATTGGGTGTCGGGTTTCCAGAGAAGCCA	ATGGTGCTGCGGTGCTGCGCAGGGGCCCGAGC
	B	GATCAGGACGAAGTCCCAGGTCCCGGACGGGG	CACCCACCCGGACTCAGAGTCTCCTCAGACGC
	C	GGCGCAGCCTGGGGGTCTCTCCCTGGTTTCCAC	TGTTTAAAGGTTTGATTCCAGCTTTTCTGAGTCCTT
	DRB1	TTATTATGAATCTCTTTTAACCTTTCTATACTTG	GGTGAGAGCTTCACAGTGCAGCGGCGAGTCC
Friend	**Locus**	**Sequence 1**	**Sequence 2**
	A	ATGGTGCTGCGGTGCTGCGTAGGGGCCCGGAC	CTCCCCAGACGCCGAGGATGGCCGTCATGGCG
	B	AACCTATGTCGGGTCCTTCTTCCAGGATACTCGTG	CACCCACCCGGACTCAGAGTCTCCTCAGACGC
	C	GGGAGGGAAACGGCCTCTGCGGAGAGGAGCGA	TGTTTAAAGGTTTGATTCCAGCTTTTCTGAGTCCTT
	DRB1	ATGGTGTGTCTGAGGCTCCCTGGAGGCTCCTG	GGTGAGAGCTTCACAGTGCAGCGGCGAGTCC
Sister	**Locus**	**Sequence 1**	**Sequence 2**
	A	ACGGAGCTCGTGGAGACCAGGCCTGCAGGGGA	ATGGTGCTGCGGTGCTGCGCAGGGGCCCGAGC
	B	AACCTATGTCGGGTCCTTCTTCCAGGATACTCGTG	CACCCACCCGGACTCAGAGTCTCCTCAGACGC
	C	GGGAGGGAAACGGCCTCTGCGGAGAGGAGCGA	TGTTTAAAGGTTTGATTCCAGCTTTTCTGAGTCCTT
	DRB1	TATTTTTCCCCAGCTATGTTGTTATCATTTCCA	GGTGAGAGCTTCACAGTGCAGCGGCGAGTCC
Husband	**Locus**	**Sequence 1**	**Sequence 2**
	A	ATGGCCGTCATGGCGCCCCGAACCCTCGTCCTG	ATGGTGCTGCGGTGCTGCGCAGGGGCCCGAGC
	B	CACCCACCCGGACTCAGAATCTCCTCAGACGCC	ATGCGGGTCACGGCGCCCCGAACCGTCCTCCT
	C	AAAGGGTGGGAGGCAGGGAGTCCAGTTCAGGGA	CGCAGCCTGGGGGTCTCTCCCTGGTTTCCACAG
	DRB1	TATTTTTCCCCAGCTATGTTGTTATCATTTCCA	TCAATTATTAAAATTAATTCTAGCTACTCTGTGG
Good Samaritan	**Locus**	**Sequence 1**	**Sequence 2**
	A	CTCCCCAGACGCCGAGGATGGCCGTCATGGCG	ATGGTGCTGCGGTGCTGCGCAGGGGCCCGAGC
	B	AACCTATGTCGGGTCCTTCTTCCAGGATACTCGTG	CACCCACCCGGACTCAGAGTCTCCTCAGACGC
	C	GGGAGGGAAACGGCCTCTGCGGAGAGGAGCGA	TGTTTAAAGGTTTGATTCCAGCTTTTCTGAGTCCTT
	DRB1	TATTTTTCCCCAGCTATGTTGTTATCATTTCCA	GGTGAGAGCTTCACAGTGCAGCGGCGAGTCC

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Module III: HLA Typing, continued

Individual	HLA-A	HLA-B	HLA-C	DRB1
Patient	A02:01 / A11:01	B44:02 / B7:527	C12:143 / C6:02	DRB1 14:84 / DRB1 08:32
Brother				
Friend				
Sister				
Husband				
Good Samaritan				

Donor → Recipient	A Matches	B Matches	C Matches	DR Matches	Total (0–8)
Brother					
Friend					
Sister					
Husband					
Good Samaritan					

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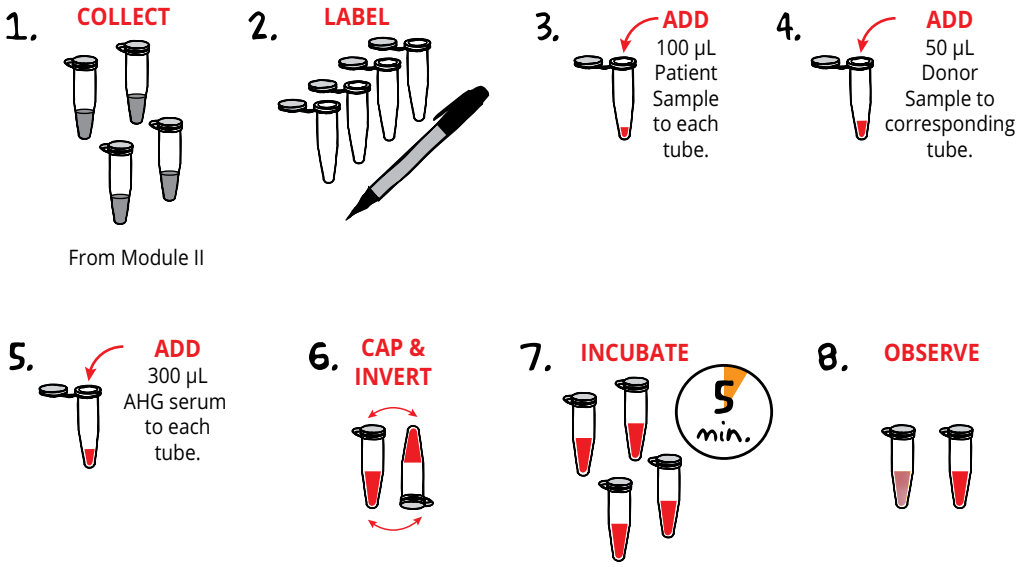
Module IV: Crossmatching



1. **COLLECT** your remaining blood samples from Module II.
2. **LABEL** four empty 1.5 mL tubes with the first letter of the samples that had **greater than a 4/8 HLA match.**
3. **ADD** 100 µL of the patient sample to each of the labeled tubes.
4. **ADD** 50 µL of each donor blood sample to appropriately labeled tube.
5. **ADD** 300 µL of Anti-Human Globulin (AHG) serum top each tube.
6. **CAP** each tube and **INVERT** several times to mix.
7. **INCUBATE** tubes at room temperature for five minutes.
8. **OBSERVE** the tubes for the presence of agglutination. Agglutination has occurred when the solution becomes cloudy and opaque. This indicates a positive result. A negative result cross match will have no agglutination.
9. **RECORD** your results below or in your lab notebook.

Sample				
positive/negative				

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Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Given the information from all the tests you performed, who is the best candidate for being the stem cell donor?
2. What is Leukemia and how is it classified?
3. Describe the eight possible ABO Rh blood types one can have and what distinguishes them.
4. What would happen if doctors were to use blood that had a positive crossmatch?
5. What is a haploidentical HLA match and why does it occur?

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Instructor's Guide

NOTES TO THE INSTRUCTOR

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK website. In addition, Technical Service is available from 8:00 am to 5:30 PM, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Need Help? Contact EDVOTEK Technical Support

Phone: 1.800.EDVOTEK
Hours: Mon–Fri, 8:00 AM – 5:30 PM EST

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- Product number and description
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Explore resources and order online:
- Online catalog
- Order products
- Experiment protocols
- Tech support
- Additional resources

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Pre-Lab Preparations

MODULE I PREP

For Module I, each student group will require a simulated white blood cell counting blood sample and one counting chamber. The instructors will dispense into snap top tubes.

1. **LOCATE** the tube labeled Simulated White Blood Cell Counting Blood Sample (Component A).
2. **INVERT** the tube multiple times to suspend simulated white blood cells
3. **ALIQUOT** 100 µL of blood solution into 10 tubes labeled "patient blood sample". ***NOTE: Every few tubes, repeat step two to make sure the "white blood cells" are thoroughly suspended for each sample.***

FOR MODULE II, Each Group Requires:

- 1 Microtiter Strip
- 1 Tube of Each P, S, B, H, F, and GS samples
- 1 Tube of each Anti-A, Anti-B, and Anti-Rh samples

MODULE II PREP

For Module II, each student group will require simulated blood sample (Patient, Sister, Brother, Husband, Friend, Good Samaritan).

1. **LOCATE** the bottles labeled Simulated Blood Sample (Type A), Simulated Blood Sample (Type B), Simulated Blood Sample (Type AB), and one empty bottle labeled Simulated Blood Sample (Type O).
2. Carefully **REMOVE** the top of the Simulated Blood Sample Type (AB) and set it aside. **ADD** 250 µL of Red dye concentrate to the bottle. **RECAP** the bottle and then gently **SHAKE** to mix.
3. Carefully **REMOVE** the top of the Simulated Blood Sample (Type B) and set it aside. **ADD** 100 µL of Red dye concentrate to the bottle. **RECAP** the bottle and then gently **SHAKE** to mix.
4. **REPEAT** step three for the remaining blood sample (Type A).
5. Carefully **REMOVE** the top of the Simulated blood Sample (Type O) bottle and set it aside. **ADD** 3.5 mL of distilled water to the bottle. **ADD** 100 µL of Red dye concentrate to the bottle. **RECAP** the bottle and then gently **SHAKE** to mix.

Aliquoting the Blood Sample

1. **LOCATE** the bags of 1.5 tubes of that will be used for distributing the blood samples.
2. **LABEL** 10 tubes "P" for the patient sample, 10 tubes "S" for sister, 10 tubes "B" for brother, 10 tubes "H" for husband, 10 tubes "F" for friend and 10 tubes "GS" for Good Samaritan.
3. **ADD** 700 µL of from the Simulated blood sample (type AB) bottle to each tube labeled P.
4. **ADD** 250 µL of the simulated blood solution (Type AB) to the 1.5 mL tubes labeled S and H.
5. **ADD** 250 µL of the simulated blood solution (Type A) to the 1.5 mL tubes labeled B.
6. **ADD** 250 µL of the simulated blood solution (Type B) to the 1.5 mL tubes labeled F.
7. **ADD** 250 µL of the simulated blood solution (Type O) to the 1.5 mL tubes labeled GS.

Aliquoting the Anti-A, Anti-B, and Anti-Rh Serum Samples

1. **LABEL** ten 1.5 mL tubes labeled with "Anti-A". Using the Anti-A bottle **ADD** 350 µL of Anti-A serum (Component F) to each tube.
2. **LABEL** ten 1.5 mL tubes labeled with "Anti-B". Using the Anti-B bottle **ADD** 350 µL of Anti-B serum (Component G) to each tube.
3. **LABEL** ten 1.5 mL tubes labeled with "Anti-Rh". Using the Anti-Rh bottle **ADD** 350 µL of Anti-Rh serum (Component H) to each tube.

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Pre-Lab Preparations, continued

MODULE IV PREP

Students will use the remaining blood samples from Module II to complete this portion of the experiment.

FOR MODULE IV, Each Group Requires:

- Blood samples from Module II
- 1 Tube of AHG sample
- 4 empty 1.5 mL tubes

Aliquoting the Simulated AHG Serum

1. **LABEL** ten 1.5 mL tube AHG.
2. **ALIQUOT** 1.4 mL of simulated AHG serum (Component I) to each tube.
3. **DISTRIBUTE** one tube of AHG and four empty 1.5 mL tubes to each group.

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Expected Results

MODULE I

Students should get a count >50,000 indicating leukemia. If the number is lower, the solution was most likely not mixed thoroughly causing the simulated white blood cells to settle.

MODULE II

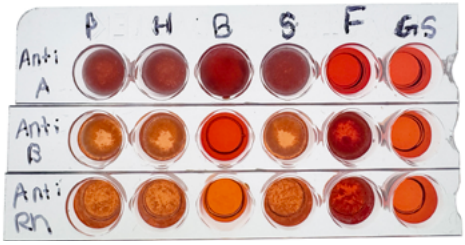
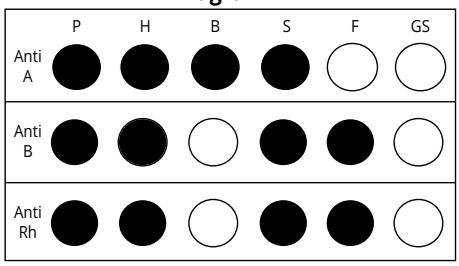


Diagram 1



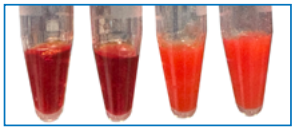
Sample	Patient	Husband	Brother	Sister	Friend	Good Samaritan
Blood Type	AB+	AB+	A-	AB+	B+	O-

MODULE III: HLA TYPING

Individual	HLA-A	HLA-B	HLA-C	DRB1
Patient	A02:01 / A11:01	B44:02 / B7:527	C12:143 / C06:02	DRB1 14:84 / DRB1 08:32
Brother	A01:01 / A11:01	B08:01 / B7:527	C03:03 / C06:02	DRB1 03:19/ DRB1 08:32
Friend	A026:254 / A03:01	B44:02 / B7:527	C12:143 / C06:02	DRB1 14:28/ DRB1 08:32
Sister	A02:01 / A11:01	B44:02 / B7:527	C12:143 / C06:02	DRB1 14:84 / DRB1 08:32
Husband	A24:02 / A11:01	B35:01 / 15:01	C07:02 / C4:01	DRB1 14:84 / DRB1 04:90
Good Samaritan	A03:01 / A11:01	B44:02 / B7:527	C12:143 / C06:02	DRB1 14:84 / DRB1 08:32

Donor → Recipient	A Matches	B Matches	C Matches	DR Matches	Total (0–8)
Brother	1	1	1	1	4
Friend	0	2	2	1	5
Sister	2	2	2	2	8
Husband	1	0	0	1	2
Good Samaritan	1	2	2	2	7

MODULE IV: CROSSMATCHING



Sample	Sister	Brother	Friend	Good Samaritan
positive/negative	negative	negative	positive	positive

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Please refer to the kit insert for the Answers to Study Questions