Edvo-Kit # 146

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Matchmakers in Medicine: Kidney Transplant Compatibility

Experiment Objective:

In this experiment, students will take on the role of transplant physicians and perform immunological testing to screen potential donors for health eligibility. Potential donors will have their blood type tested and undergo a crossmatch to see if their immune system is compatible with the patient. Students will then analyze the results to determine the best possible donor for the transplant.

See page 3 for storage instructions.

Version 146.250930

SAMPLE LITERATURE
Please refer to included weblink for correct version

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Experiment Components

This experiment is designed for 10 groups.

Component

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

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)*

Supplies	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

WHAT IS PKD?

Polycystic Kidney Disease (PKD) is a chronic, inherited condition that causes fluid-filled sacs called cysts to form in the kidneys. These cysts grow over time and make it harder for the kidneys to function properly. Healthy kidneys filter waste, balance fluids, and help regulate blood pressure. But in PKD, the growing cysts take up space and damage the kidney tissue, making it harder for the kidneys to do their job (Figure 1) .

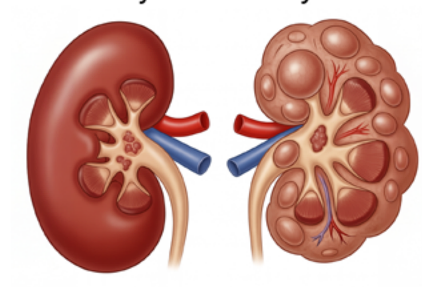

Figure 1:
Comparison of a normal kidney verses a kidney suffering from Polycystic Kidney Disease.

PKD is one of the most common life-threatening genetic diseases. It affects people of all ages and backgrounds, and many don't even know they have it until symptoms appear later in life.There are two main types of PKD:

1. **Autosomal Dominant PKD (ADPKD)**
   - The most common form.
   - Usually shows symptoms between ages 30 and 50, but cysts can begin developing in childhood.
   - If one parent has ADPKD, there is a 50% chance it will be passed to their children. Symptoms include high blood pressure, back or side pain, kidney stones, and blood in the urine.

2. **Autosomal Recessive PKD (ARPKD)**
   - Much rarer and more serious.
   - Usually diagnosed in infants or even before birth.
   - Both parents must carry the gene for a child to inherit the condition.
   - Can cause enlarged kidneys at birth, trouble breathing, and liver problems.

PKD mainly affects the kidneys, but it can also impact other parts of the body. The cysts in the kidneys can grow so large and numerous that the kidneys become enlarged—sometimes up to four times their normal size. As a result, kidney function declines. PKD is often diagnosed with imaging tests, such as ultrasound, CT scan, or MRI that can see the cysts.Doctors also use blood tests to check kidney function and urine tests to look for signs of blood or protein.

There is no cure for PKD yet, but treatment can help manage symptoms and slow kidney damage. Common treatments include: blood pressure medications, pain relief medications for kidney or back pain, antibiotics to treat any infections that arise, diet changes, and regular kidney monitoring. Eventually, some people with PKD develop kidney failure, which means their kidneys can no longer filter waste well enough to keep them healthy. This stage is also called end-stage renal disease (ESRD).

When kidneys fail, people need renal replacement therapy, which means either: dialysis – A machine filters your blood for you or a kidney transplant. While dialysis can keep people alive, a kidney transplant is often the best option for long-term health and quality of life.

A kidney transplant is a surgery to place a healthy kidney from a donor into a person with kidney failure. The new kidney does the work that the failing kidneys can no longer do.

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There are two types of donors:

1. **Living Donor**
    - A friend, relative, or someone else who donates one of their kidneys.
    - People can live healthy lives with just one kidney

2. **Deceased Donor**
    - A kidney donated by someone who has died and chosen to donate their organs.

Before the transplant, doctors run many tests to make sure the donor and recipient are a good match. This includes tests on the donor and recipient blood such as blood typing, HLA matching and crossmatching. A good match reduces the risk that the recipient's body will reject the new kidney.

Kidney Donor Screening

Screening potential kidney donors is a thorough, multi-step process that ensures both safety and compatibility before transplantation. It begins with a detailed medical history review and physical examination, where past illnesses, surgeries, and family medical history are carefully evaluated to rule out hidden risks. Immunological testing follows, including an ABO blood type test and HLA tissue typing, which identifies genetic markers on white blood cells to measure donor–recipient compatibility. A crossmatch test is often performed to see if the recipient's immune system produces antibodies that would attack the donor's cells.

Beyond immunological factors, donors are extensively screened to ensure they are good candidates. In order to donate a kidney, donors must undergo:

- **Laboratory Blood Tests**
    - Complete blood count (CBC) for red and white cells.
    - Kidney function: creatinine, BUN, and GFR.
    - Electrolytes and glucose tolerance (screen for diabetes risk).
    - Liver and pancreas function tests.
    - Infectious disease screening: HIV, hepatitis B & C, CMV, syphilis.
    - Test for past viral activity that could reactivate after transplant.

- **Urine Testing**
    - 24-hour urine collection for creatinine clearance (kidney function).
    - Check for protein, blood, or signs of infection.
    - Imaging & Cardio-Pulmonary Tests
    - Chest X-ray: Assess lung health.
    - EKG: Assess heart rhythm and function.
    - Intravenous pyelography: Dye study to examine kidneys and urinary tract.
    - CT scan or MRI: Look for cysts, tumors, or anatomical issues.

- **Additional Testing for Women**
    - Gynecological exam and mammogram to ensure reproductive health is clear.

- **Psychological Evaluation**
    - Confirm motivation and voluntary choice.
    - Provide emotional support.
    - Ensure no outside pressure is influencing the decision..

Together, these comprehensive laboratory and medical tests confirm that the donor is healthy, safe to undergo surgery, and offers the best chance for a successful transplant.

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IMMUNOLOGICAL TESTING

Blood Typing

During a donor blood screening the blood undergoes ABO and Rh typing to confirm a donors blood type. 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, 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.

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 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 2)**. A haploidentical match means that the donor and recipient share exactly half (50%) of their

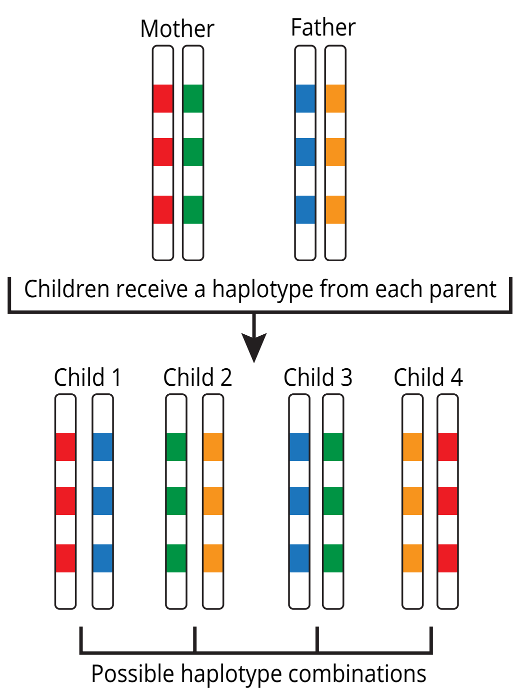

Figure 2:
Parents and children are haploidentical.

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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.

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 3)**.

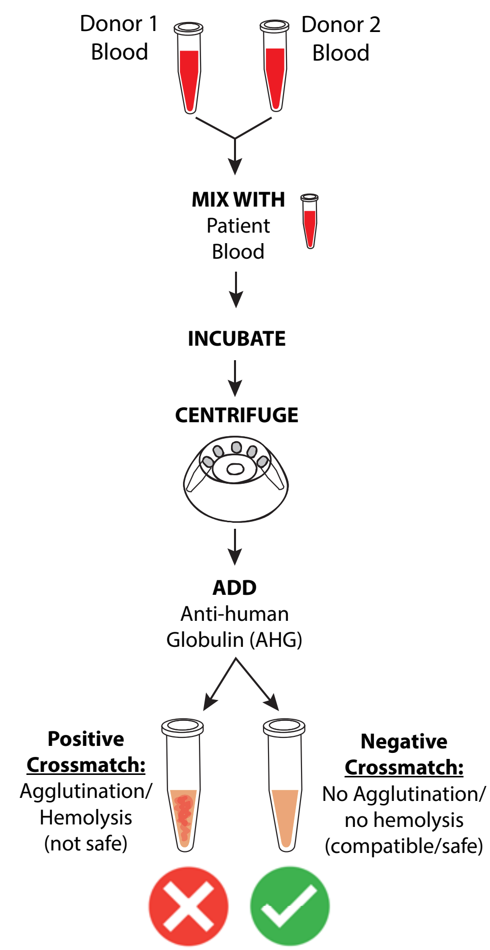

Figure 3: Process of blood crossmatching.

AFTER THE TRANSPLANT

After receiving a kidney, the patient must take immunosuppressant medications. These drugs keep the immune system from attacking the new kidney. Without them, the body would see the new kidney as foreign and try to destroy it.

Post-transplant care includes:
- Regular checkups
- Blood tests to monitor kidney function
- Following a healthy lifestyle
- Taking medications exactly as prescribed

Doctors also monitor the patient closely to make sure the new kidney is working properly and to watch for any signs of rejection. One important way they do this is by looking at the patient's white blood cell count.

White blood cells (WBCs) are part of the immune system. They help defend the body against infections, cancer, and anything the body sees as "foreign," including transplanted organs. If the immune system starts to reject the new kidney, it often becomes more active—and that can lead to a rise in the number of white blood cells.

A high white blood cell count after a transplant may be a sign of:
- Infection (because the immune system is responding)
- Organ rejection (because the immune system is attacking the new kidney)
- Medication Side Effects: Some drugs used after transplant (such as corticosteroids) can cause higher WBC counts as a side effect.
- Inflammation or Stress Response: Surgery, inflammation, or even physical stress on the body can sometimes keep WBC counts elevated.

Doctors often order a WBC count as part of routine blood work. This test measures the number of white blood cells in a microliter (μL) of blood. While some changes are normal, a significant increase can be a warning sign that the immune

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system is overactive, and doctors may need to adjust medications or do more tests.

Sometimes, if there are signs of rejection, doctors may also do:
- A kidney biopsy – taking a tiny piece of kidney tissue to check under a microscope.
- Other lab tests – to check for kidney function, like creatinine and blood urea nitrogen (BUN) levels.

By regularly monitoring white blood cell counts and other lab results, doctors can catch signs of rejection early and take steps to protect the new kidney.

THE SCENARIO:

Talia is a 16-year-old girl living with polycystic kidney disease (PKD), a genetic condition she inherited from her mother. Talia's mother knew there was a high chance that one of her kids would inherit her disease and therefore has monitored Talia since she was very young. Six years ago after experiencing a rapid onset of symptoms, Talia was officially tested and diagnosed with PKD. Although rare in children her age, Talia faced a swift decline over the past six years and despite years of treatment, her doctors recently explained that her kidneys are no longer working well enough, and she will soon need a kidney transplant to survive.

Her immediate family steps forward to be tested for compatibility. After blood type and HLA testing, a few relatives are found to be potential matches. However, the doctors recommend also exploring additional donor options to ensure the best possible outcome for Talia and to have backup options in case of complications.

They begin reaching out to friends, neighbors, and members of their community, explaining what PKD is, how kidney donation works, and how one healthy kidney could give Talia the chance to live a long, healthy life. Soon, people from their school and local community come forward to be tested, showing how collective support can play a role in finding the right match for Talia.

In this activity, students take on the roles of Talia's transplant team and perform immunological testing to screen potential donors for health eligibility. The donors will then have their blood type tested for verification of donor-reported blood type and undergo a crossmatch to see if their immune system is compatible with Talia's. Students will then analyze the results to determine the best possible donor for the transplant.

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Additional Activity

For further learning into Talia's condition, students can connect two different laboratory experiments: a urine screening and a blood typing test. **EDVO-Kit #145: Tracking Health Through Urinealysis: A Case-based Clinical Simulation**. Together, these activities model the kinds of investigations that doctors might use when evaluating a patient with polycystic kidney disease.

The urine screening experiment helps students model how doctors would investigate her symptoms. By analyzing simulated urine samples, students look for abnormalities such as the presence of protein. Proteinuria often occurs when kidney filters are damaged, as in PKD, and serves as a critical warning sign of declining kidney health. In Talia's case, these results could suggest that her symptoms are linked to her family history and that her kidneys may not be functioning properly.

The blood typing experiment shifts focus to the challenges Talia would face if she ultimately needs a transplant. Determining her ABO and Rh blood type is one of the first steps in identifying potential donors. Blood type testing ensures that her immune system will not immediately reject a transplanted kidney. If her type is compatible with a family member or community donor, this would guide the next stages of screening, including tissue typing and crossmatching.

By using Talia as the patient for both activities, students can see how different tests connect in the bigger picture of kidney disease and transplantation. Urine testing shows whether the kidneys are healthy enough to do their job, while blood typing highlights how donor and recipient must be immunologically compatible. Together, these experiments give students a realistic picture of how clinicians begin evaluating patients with kidney disease and prepare for the possibility of transplantation.

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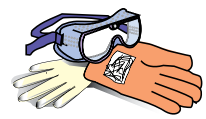
Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will take on the role of transplant physicians and perform immunological testing to screen potential donors for health eligibility. Potential donors will have their blood type tested and undergo a crossmatch to see if their immune system is compatible with the patient. Students will then analyze the results to determine the best possible donor for the 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: Donor Selection

In this activity, you will review a living donor registry made up of volunteers from Talia's community who are willing to donate a kidney. Your task is to screen each potential donor and decide whether they are eligible for transplant surgery based on the medical criteria provided. Use the space given to record whether each donor is eligible or not, and explain your reasoning. Important: HLA matches below 4/8 are not considered acceptable.

	Donor Name	Relationship to Talia	Blood Type	HLA Match (8/8)	Health Screening	Kidney Function	Infectious Disease Screening	Mental Health Screening
1	Garrett N.	Dad	AB+	4/8	Passed	Normal	Passed	Passed
2	Zayden N.	Older Brother	A-	8/8	Passed	Normal	Passed	Passed
3	Sloane N.	Older Sister	AB+	4/8	Passed	Normal	Passed	Passed
4	Rowan K.	Cousin	O+	3/8	Passed	Normal	Passed	Passed
5	Ophelia S.	Family Friend	AB+	2/8	Passed	Normal	Passed	Passed
6	Evie M.	Teacher	A-	6/8	Passed	Normal	Passed	Failed
7	Tristen S.	Neighbor	O-	5/8	Passed	Normal	Passed	Passed
8	Raven A.	Grandmother	B+	3/8	Passed	Normal	Passed	Passed
9	Kai G.	Local Volunteer	A+	6/8	Failed	Normal	Passed	Passed
10	Rai S.	Aunt	B+	7/8	Passed	Normal	Passed	Passed
11	Jeremy V.	Uncle	O-	8/8	Passed	Abnormal	Passed	Passed

Donor 1: ______________________________

Donor 2: ______________________________

Donor 3: ______________________________

Donor 4: ______________________________

Donor 5: ______________________________

Donor 6: ______________________________

Donor 7: ______________________________

Donor 8: ______________________________

Donor 9: ______________________________

Donor 10: ______________________________

Donor 11: ______________________________

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Module II: Confirmatory ABO Rh Blood Typing

Even if the donor already knows their blood type, testing is done again in the screening process to confirm accuracy, because mistakes in blood typing can cappen and can have serious consequences in transplantation. In this module, you will perform ABO Rh blood typing to contirm blood types and check for mistakes.

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: T, F, B, S, A, and N. These letters correspond with the donor blood samples:
   T=Talia, F=Father, B=brother, S=sister, A=Aunt, and N=Neighbor.

   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.

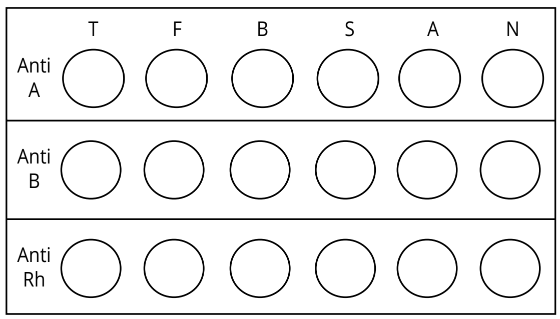
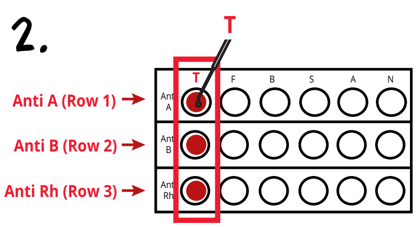
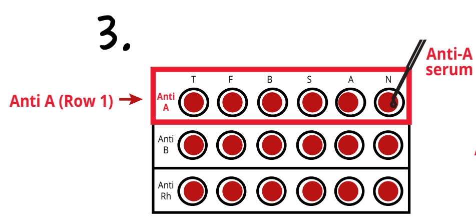


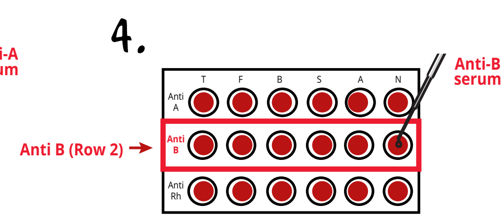
Diagram 1



ADD 50 µL of blood sample "T" 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.

2. **ADD** 50 µL (or two drops from a transfer pipet) of blood sample "T" into each of the its three corresponding wells under the letter "T". **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.

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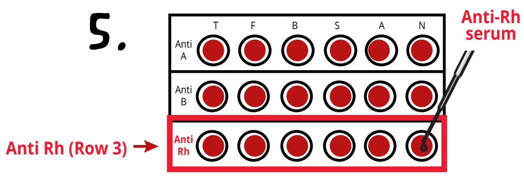
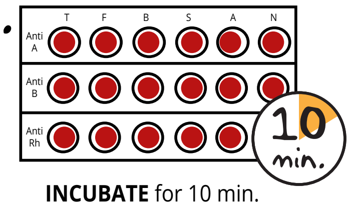
Module II: Confirmatory ABO Rh Blood Typing, continued

5.



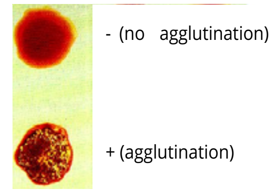
ADD 50 µL of Anti-Rh serum into all wells in Row 3.

6.



INCUBATE for 10 min.

7.



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 III.

Sample	Talia (T)	Father (F)	Brother (B)	Sister (S)	Aunt (A)	Neighbor (N)
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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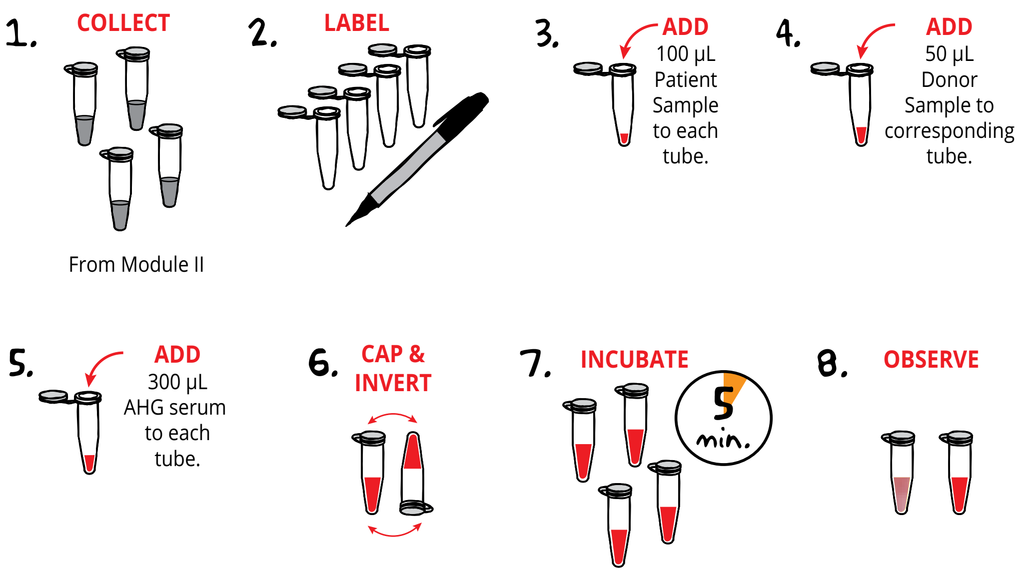
Module III: Crossmatching



1. **COLLECT** your remaining blood samples from Module II.
2. **LABEL** four empty 1.5 mL tubes with the first letter of the samples from Module II.
3. **ADD** 100 µL of Talia's 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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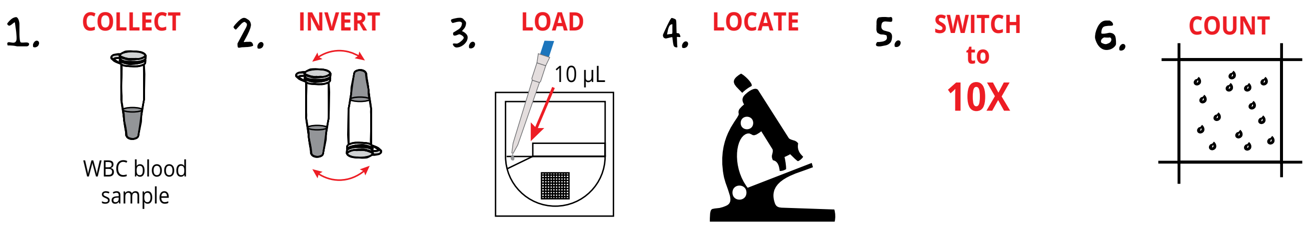
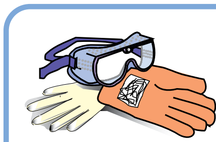
Module IV: White Blood Cell Count




Wear gloves and safety goggles

1. **OBTAIN** Talia's WBC blood sample
2. **INVERT** for several seconds to make sure the white blood cells are suspended
3. **LOAD** 10 µL of Talia's WBC 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 >11,000 is considered high. ***NOTE: the blood sample used in this activity have been diluted by a factor of 100***

WBC/µL= (average number of cells per grid) x (dilution factor) / volume of sample in grid (10 µL)

Grid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Cells									

Average:______________________________

Number of white blood cells per µL: ______________________________

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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 kidney donor?

2. What is Polycystic Kidney Disease (PKD) and how does it affect the kidneys?

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

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Pre-Lab Preparations

MODULE II PREP

For Module II, each student group will require simulated blood sample (Talia, Father, Brother, Sister, Aunt, and Neighbor).

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.

**FOR MODULE II,
Each Group Requires:**

- 1 Microtiter strip
- 1 Tube of each sample: T, F, B, S, A, and N.
- 1 Tube of each Anti-A, Anti-B, and Anti-Rh samples

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 "T" for the Talia, 10 tubes "F" for father, 10 tubes "B" for brother, 10 tubes "S" for sister, 10 tubes "A" for aunt and 10 tubes "N" for neighbor.
3. **ADD** 700 µL of from the Simulated blood sample (type AB) bottle to each tube labeled "T".
4. **ADD** 250 µL of the simulated blood solution (Type AB) to the 1.5 mL tubes labeled "F".
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 "S" and "A".
7. **ADD** 250 µL of the simulated blood solution (Type O) to the 1.5 mL tubes labeled "N".

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** 750 µL of Anti-A serum (Component E) to each tube.
2. **LABEL** ten 1.5 mL tubes labeled with “Anti-B”. Using the Anti-B bottle **ADD** 750 µL of Anti-B serum (Component F) to each tube.
3. **LABEL** ten 1.5 mL tubes labeled with “Anti-Rh”. Using the Anti-Rh bottle **ADD** 750 µL of Anti-Rh serum (Component G) to each tube.

MODULE III PREP

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

**FOR MODULE III,
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 tubes "AHG" and **ALIQUOT** 1.4 mL of simulated AHG serum (Component H) to each tube.
2. **DISTRIBUTE** one tube of AHG and four empty 1.5 mL tubes to each group.

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Pre-Lab Preparations, continued

MODULE IV PREP

For Module IV, 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 J).
2. **INVERT** the tube multiple times to suspend simulated white blood cells
3. **ALIQUOT** 100 µL of blood solution into 10 tubes labeled "Talia WBC blood sample".
   NOTE: Every few tubes, repeat step two to make sure the "white blood cells" are thoroughly suspended for each sample.

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Expected Results

MODULE I

Students should identify Talia's father (Garrett), brother (Zayden), sister (Sloane), aunt (Rai), and neighber (Tristen) as the best possible donor candidates.

MODULE II

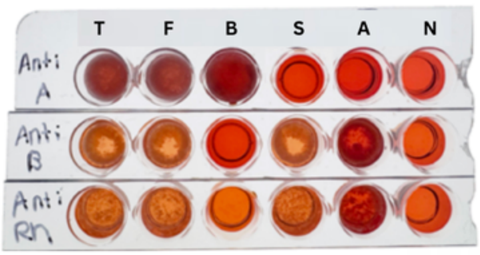
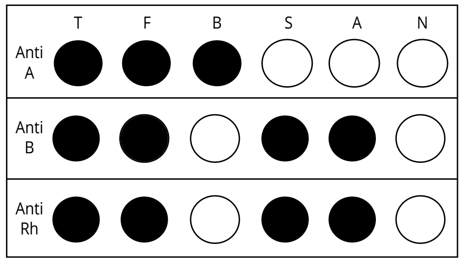


Diagram 1



Sample	Talia	Father	Brother	Sister	Aunt	Neighbor
Blood Type	AB+	AB+	A-	B+	B+	O-

MODULE III: CROSSMATCHING

Sample	Father	Brother	Sister	Aunt	Neighbor
positive/negative	negative	negative	positive	positive	positive

MODULE IV: WHITE BLOOD CELL COUNT

Students should get a count >11,000 indicating a high WBC. If the number is lower, the solution was most likely not mixed thoroughly causing the simulated white blood cells to settle.

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