

An Introduction to HLA

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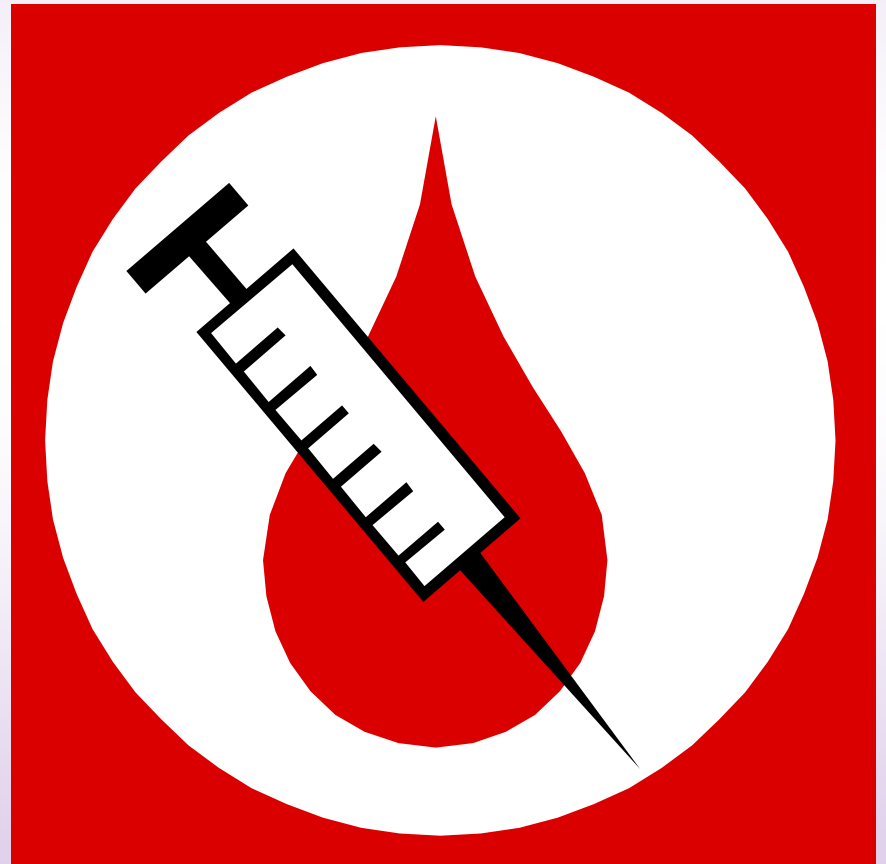


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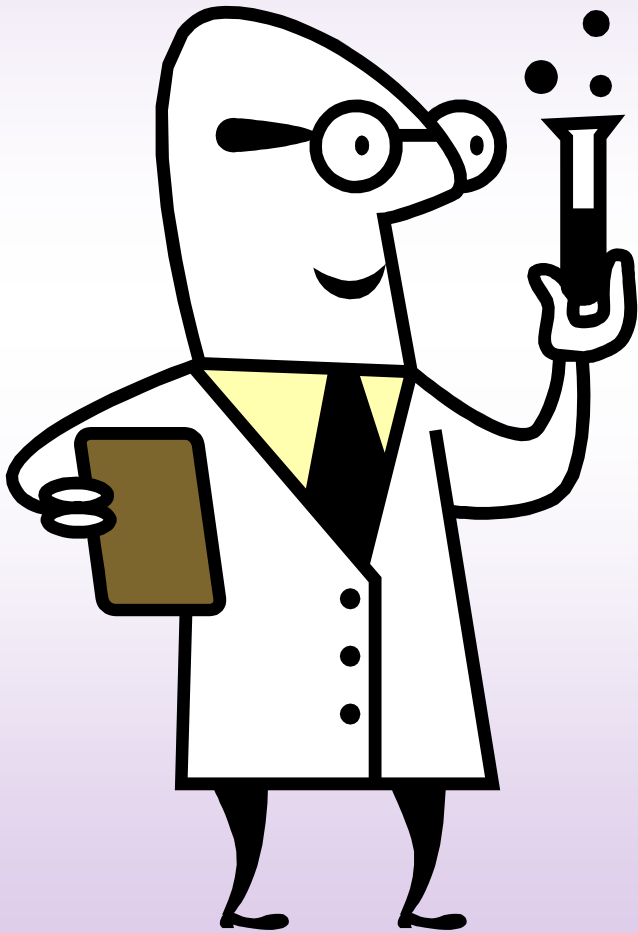
SCHOOL *of* MEDICINE

Purpose

- The purpose of this presentation is to aid understanding of the basic immunology underlying the transplantation rejection process.



Let's talk about HLA



- You may have heard of “tissue typing” or “good” matches during discussions about transplantation.
- What exactly are we talking about?
- We are talking about a set of proteins on the surface of every person's cells called HLA. HLA stands for Human Leukocyte Antigen. Let's look at each of those words to understand what they mean.

What does HLA stand for?

- **HLA = Human Leukocyte Antigen**
- **Human = human**
- **Leukocyte = white cell**
- **Antigen**
 - something the immune system reacts to
 - antigens on cell surfaces can act as flags
- **HLA**
 - flags that the immune system looks at to determine if something is an invader



Flags?

- This may be hard to think about.
- Let's do a small thought experiment to see how small changes in a protein (flag) can lead to extremely large differences in how they can be seen by the immune system.



What is this?



Click again for the answer.

What is this?



Click again for the answer.

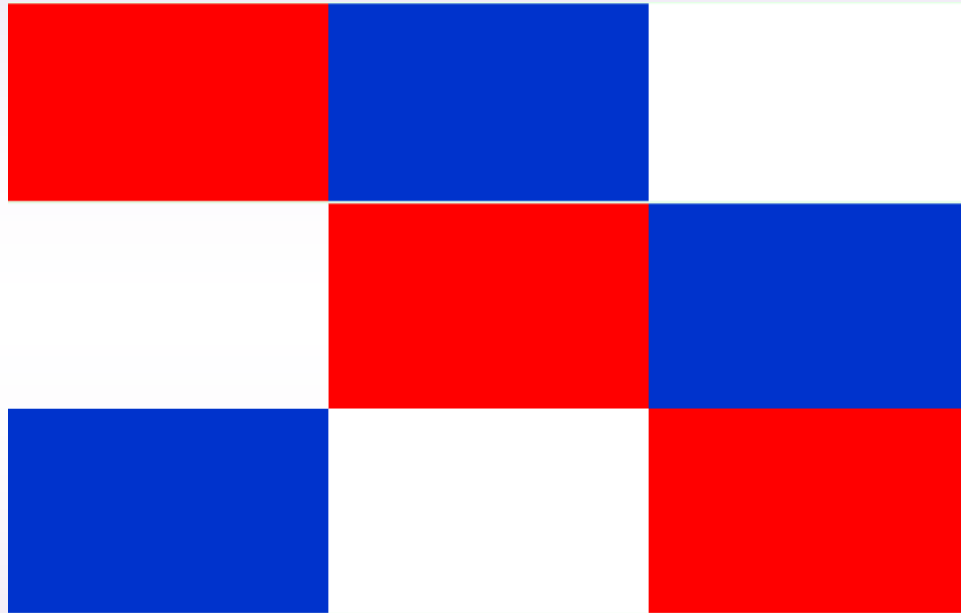
Correct, it's a rectangle!

Let's add some colors!

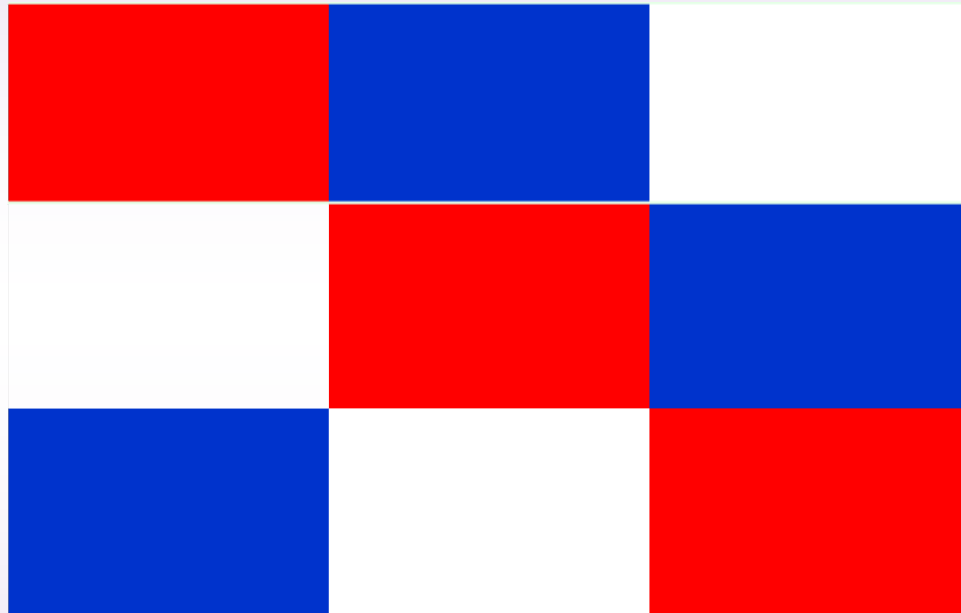


Click to add three colors

Three colors in a rectangle!

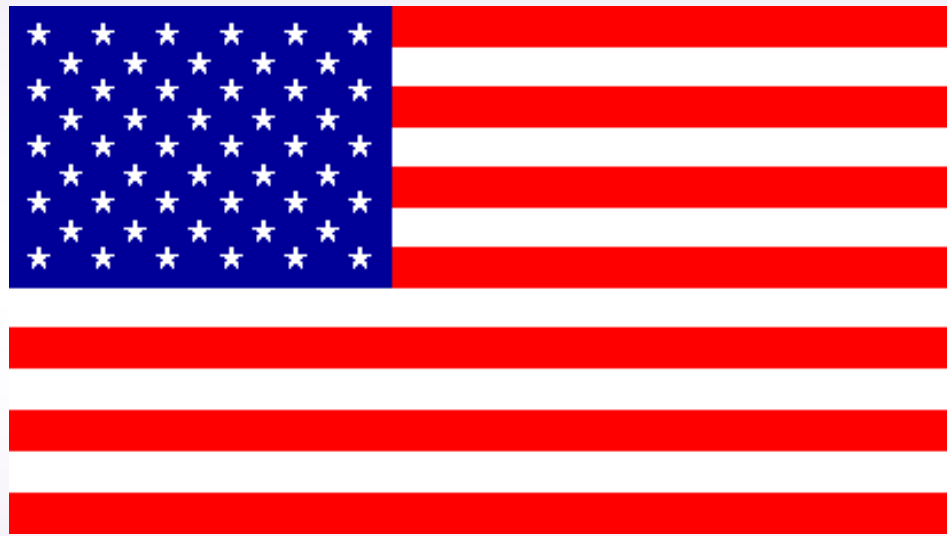


Doesn't look like much, eh?

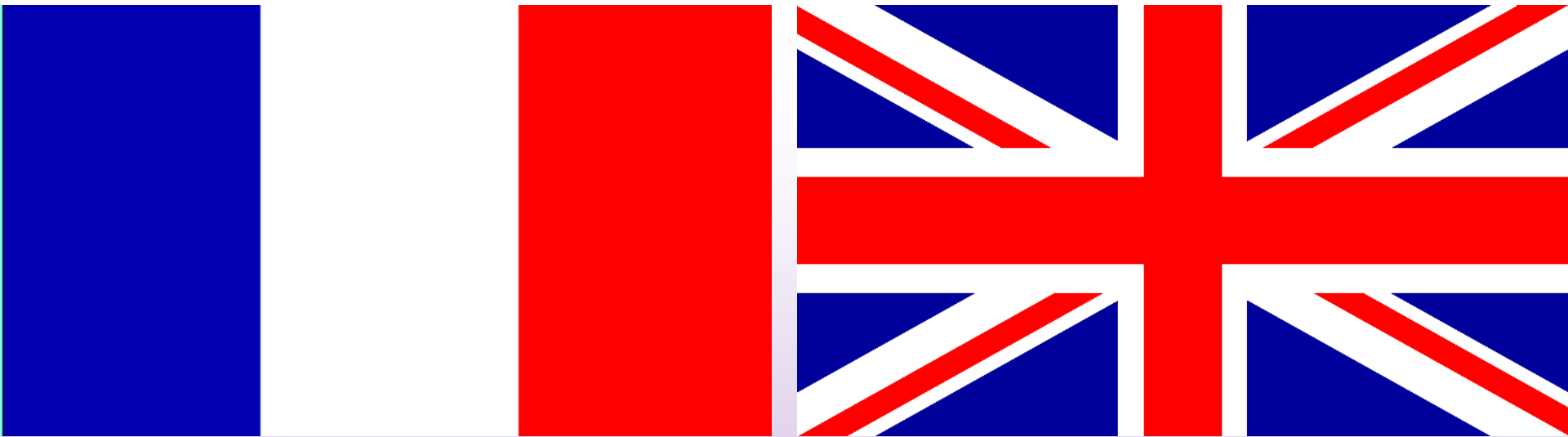
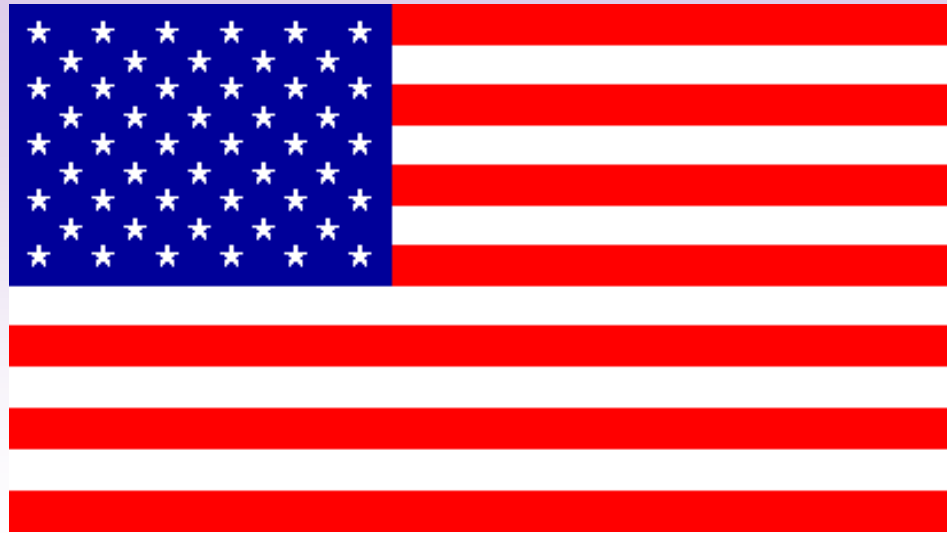


Let's rearrange these three colors.

This looks like something now!



Let's arrange these colors again, shall we?



As you can see, making changes in the three basic components can radically change how an antigen is perceived.

Remember

- The immune system looks for differences in HLA to see if there is a need to react.
- Just like a foreign army has a different flag, cells or organs from different people have different flags.
- This inspection and recognition is the heart of the transplant rejection process.

Are these friends or foes?



OK, we have flags. So what?

- **When a patient goes through the pre-transplant workup, one of the tests performed is HLA typing to determine the patient's HLA antigens (flags).**
- **When a patient is put on the transplant list, the HLA antigens are listed into a special computer system, along with several other pieces of information.**
- **When a potential donor is identified, the donor is tissue typed and the donor's HLA type is listed in the same computer system.**
- **A lot of factors play a role in determining which patient receives the donor's organ.**
 - **Special consideration may be given to people on the waiting list whose HLA type closely matches the donor's HLA type.**

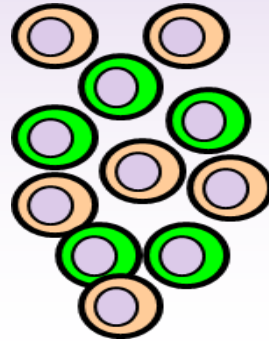
HLA Typing

- A process of determining which HLA antigens (flags) that a patient or a donor has.
- Lots of ways to do this testing
- Majority are now done by DNA methods
- How is it done?

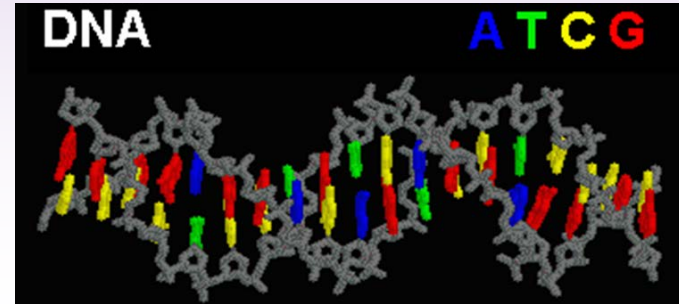
HLA Typing Process



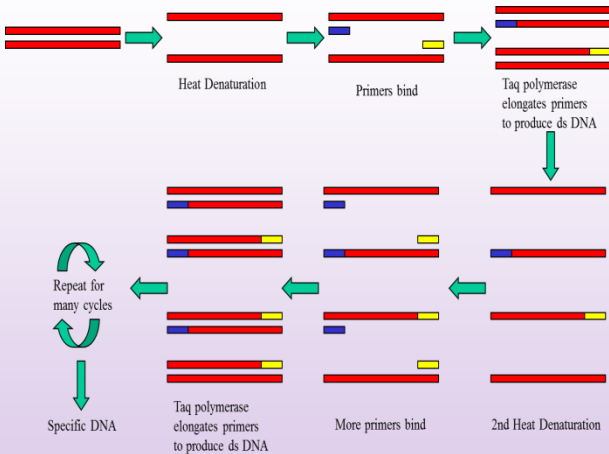
Step 1: Get blood



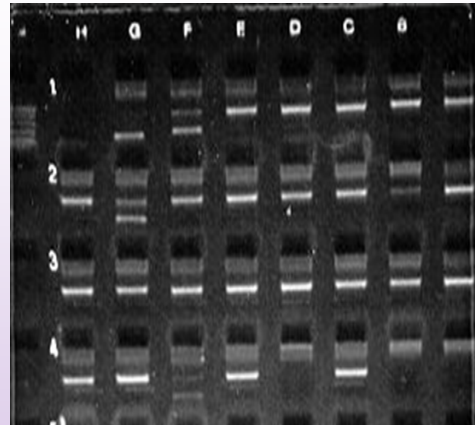
Step 2: Isolate Cells



Step 3: Isolate DNA from cells



Step 4: PCR Reactions



Step 5: Analyze Reactions



	H1	G1	F1	E1	D1	C1	B1	A1
Void Test								
DRB1*0103,0414,0701,1114/20,1								
DRB1*0412/18,0810/12								
DRB1*0801/021-022/041-042/06-09/11-12/30								
DRB1*08031-032/14-15,1313								
DRB1*0816								
DRB1*1115								
DRB1*1118,1306								
DRB1*1119,1312/30,1605								
DRB1*1124								
DRB1*1128								
DRB5*0101								
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DRB5*0203								
DRB5*0204								

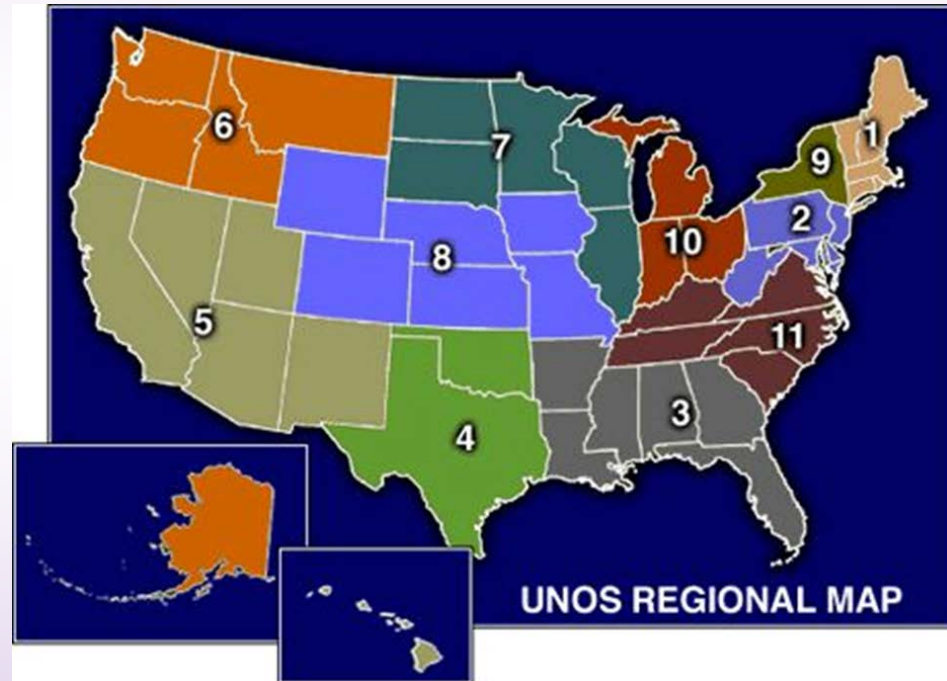
Step 6: Determine typing

That's a lot!

- **Yes it is, and no, you don't have to really know about how the testing is done.**
- **Just know that it is done by well-trained extremely motivated laboratory professionals who are not only specially trained but can be specially certified.**

What's next?

- For a kidney, pancreas, liver, heart or lung patient, the typing is placed into the national transplant list.
- The list is maintained by the **United Network for Organ Sharing or UNOS**
 - UNOS actually runs the federal Organ Procurement and Transplantation Network
 - More info at
 - » www.unos.org
 - » www.organdonor.gov

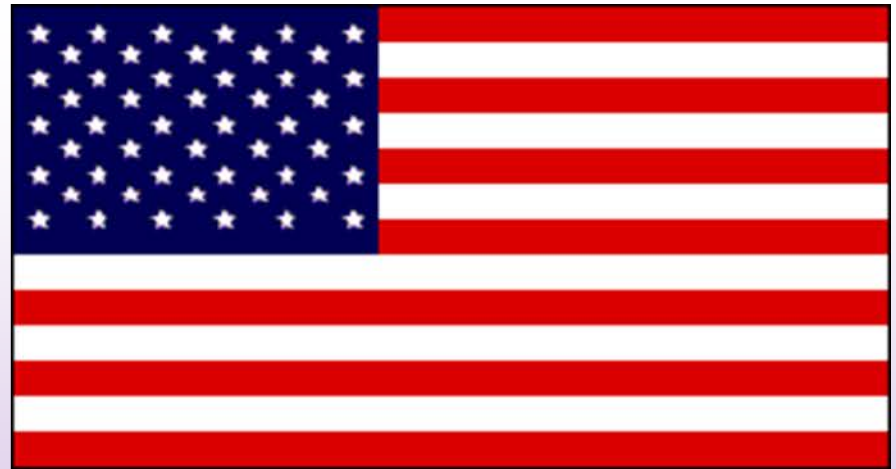
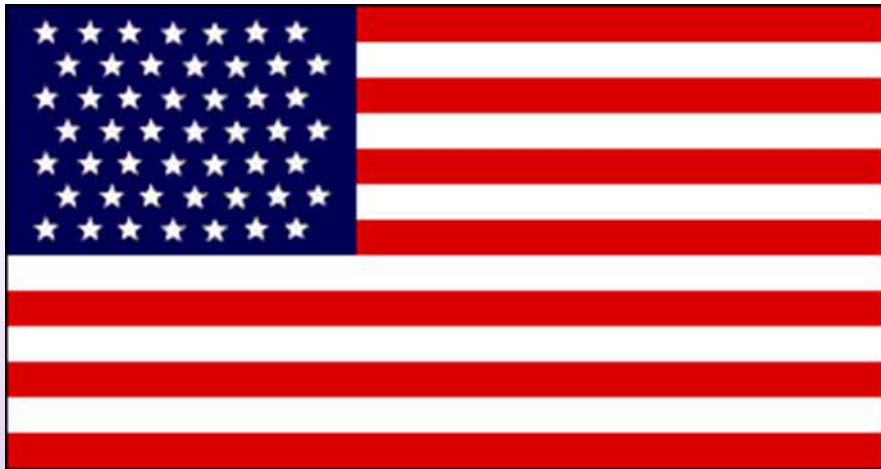


What about Bone Marrow?

- A bone marrow or cord blood transplant may be the best treatment option or the only potential for a cure for patients with leukemia, lymphoma, sickle cell anemia and many other diseases.
 - Go to bethematch.org for a lot more information
- A higher level of HLA typing is performed for bone marrow transplantation than for solid organ transplants.
- Why? Because small differences in HLA between solid organ donors and recipients are easier to treat than small differences between bone marrow donors and recipients.

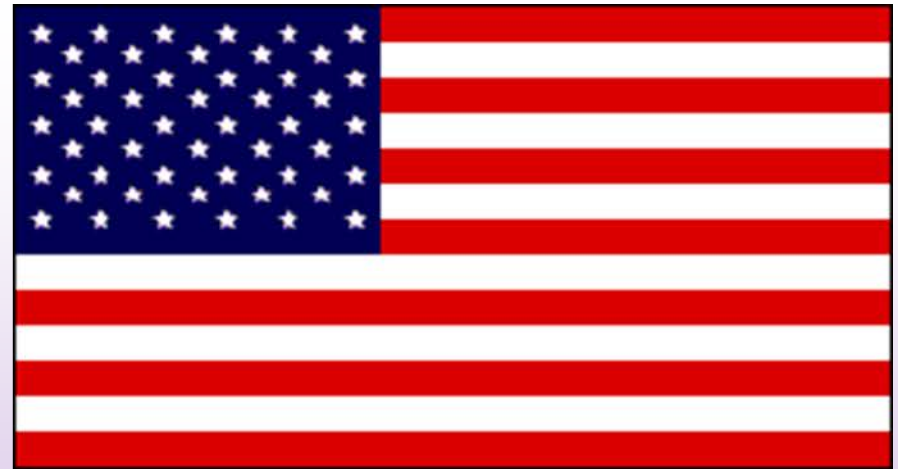
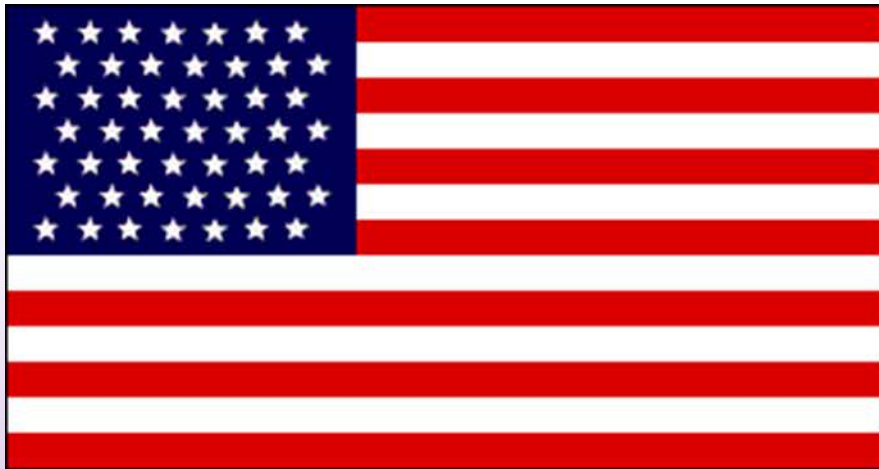
What do you mean by small differences?

- OK, let's go back to our flags.
- Can you spot the difference in these flags?
 - Click to find out



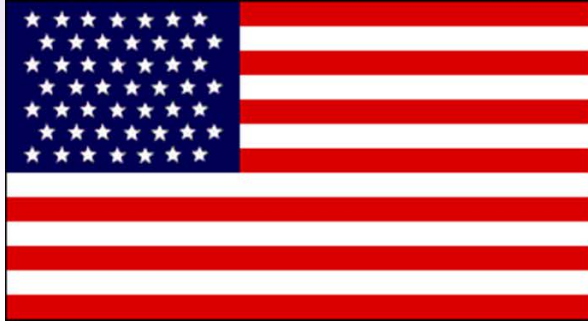
What do you mean by small differences?

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- Can you spot the difference in these flags?
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There is a difference in the number of stars!

What?



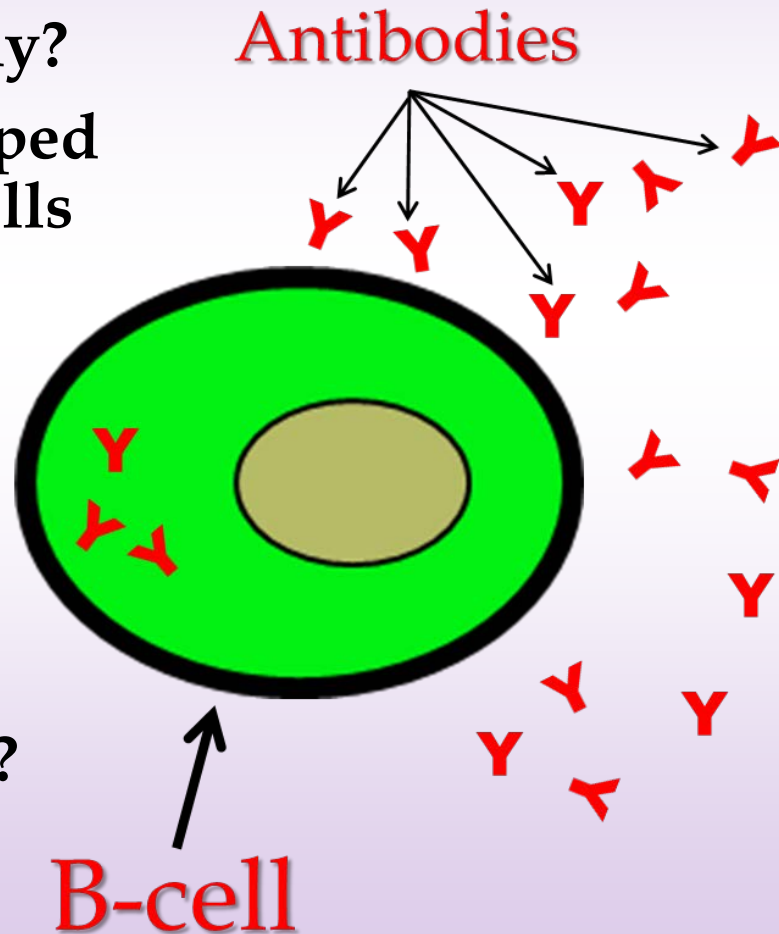
- **The flag on the left has 49 stars**
 - This was the US flag from July 4, 1959 to July 4, 1960
- **A difference as small as this one can cause a life-threatening complication in bone marrow transplantation called graft-versus-host disease.**
- **It is easier to treat rejection in solid organ transplants than it is to treat graft-versus-host disease.**

Is there more?

- **Oh, yes!**
- **There are two arms of the immune system, cell-based and antibody-based.**
- **We've only talked about cell-based.**
- **Let's talk antibodies.**

Antibodies?

- OK, what is an antibody?
- An antibody is a Y-shaped molecule made by B-cells



- What do antibodies do?

Antibodies

- Basically, antibodies bind to things. Those “things” are called antigens.
 - (Remember Antigens = Flags)
- The term antibody was first used by Paul Ehrlich in 1908.
- Other terms that mean antibodies are anti-toxins, anti-venoms, anti-globulins, immunoglobulins, etc.
- Normally, antibodies are very important in protecting us.



Paul Ehrlich

Antibodies

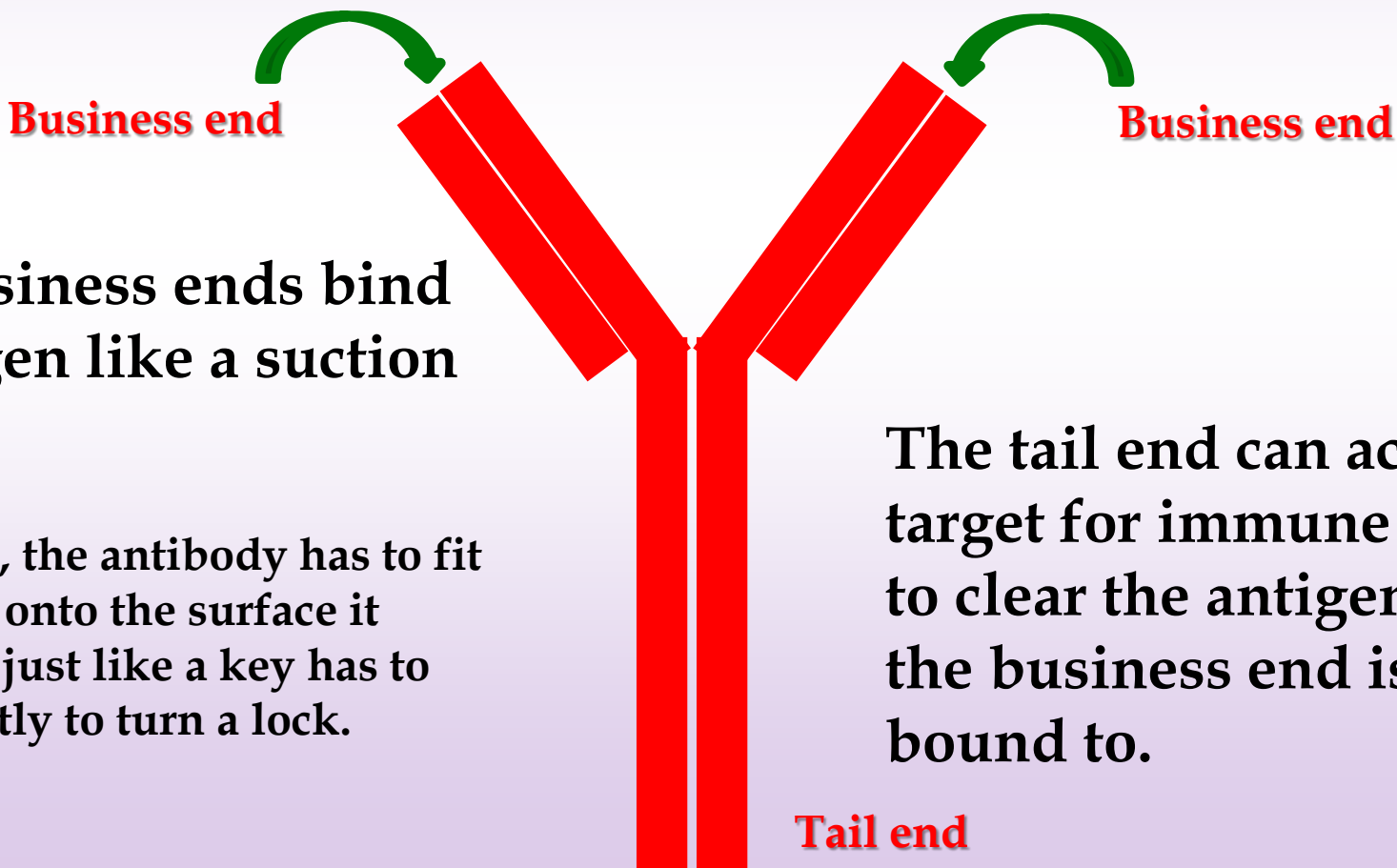
- **It cannot be stressed hard enough that antibodies are among the defenses that the body uses to repel foreign invaders.**
 - Sometimes, antibodies are thought of as “bullets” of the immune system.
- **Vaccines cause the immune system to make antibodies which protect us from infectious diseases**
 - Antibodies prevent us from getting most diseases a second time.
- **However, sometimes antibodies can be bad.**

Bad antibodies

- **Antibodies play major roles in certain autoimmune diseases**
 - Addison's disease, autoimmune hepatitis, thrombocytopenia purpura, Goodpasture's syndrome, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, and pernicious anemia (just to name a few)
- **Allergies are caused by antibodies that are attacking a fairly harmless thing like plant pollen.**
- **Hemolytic disease of the newborn is caused by antibodies from the mother attacking red blood cells of the baby while it is in the womb.**
- **Antibodies can attack an organ after it is transplanted.**

Antibody Structure

- An antibody has two “business” ends and one “tail” end.



The business ends bind to antigen like a suction cup.

In reality, the antibody has to fit perfectly onto the surface it binds to, just like a key has to fit perfectly to turn a lock.

The tail end can act as a target for immune cells to clear the antigen that the business end is bound to.

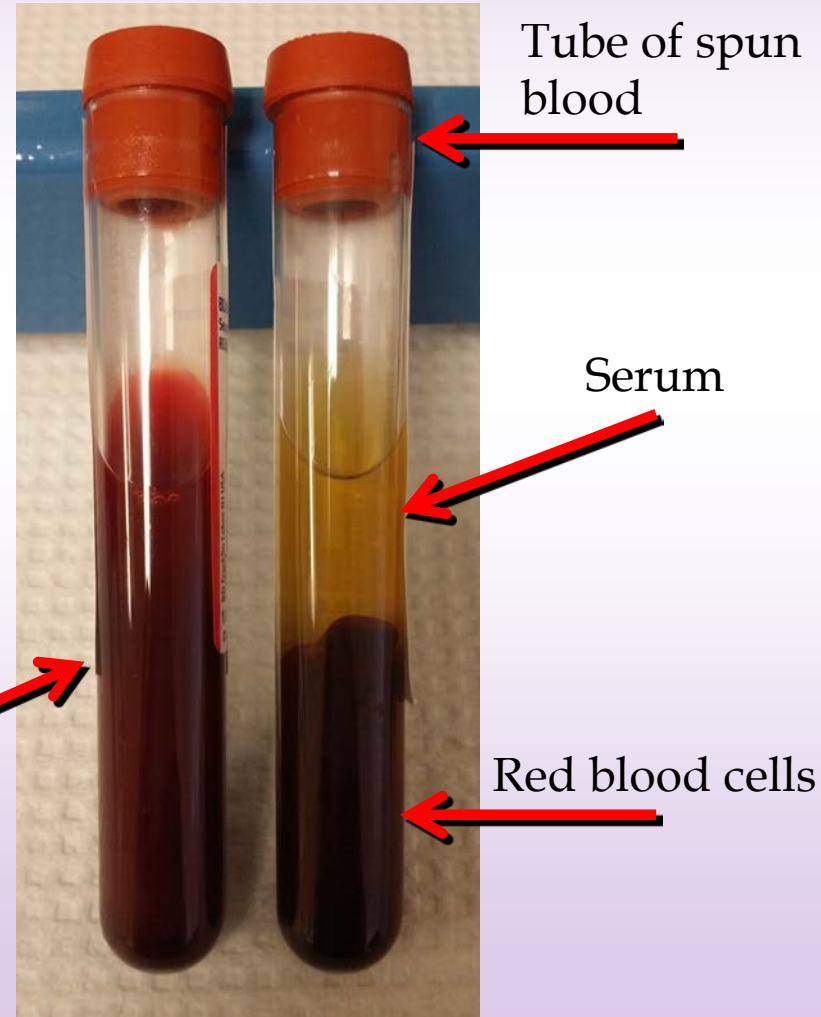
Why are we talking about this?

- **Antibodies can bind to a transplanted organ and cause the immune system to destroy it.**
- **Antibodies can also bind to the cells of a bone marrow transplant and cause it to fail.**
- **Most of the time these antibodies are directed against HLA.**
- **There are a couple of different ways to test a patient to see if they have antibody to the HLA of the donor.**

Antibodies are found in serum

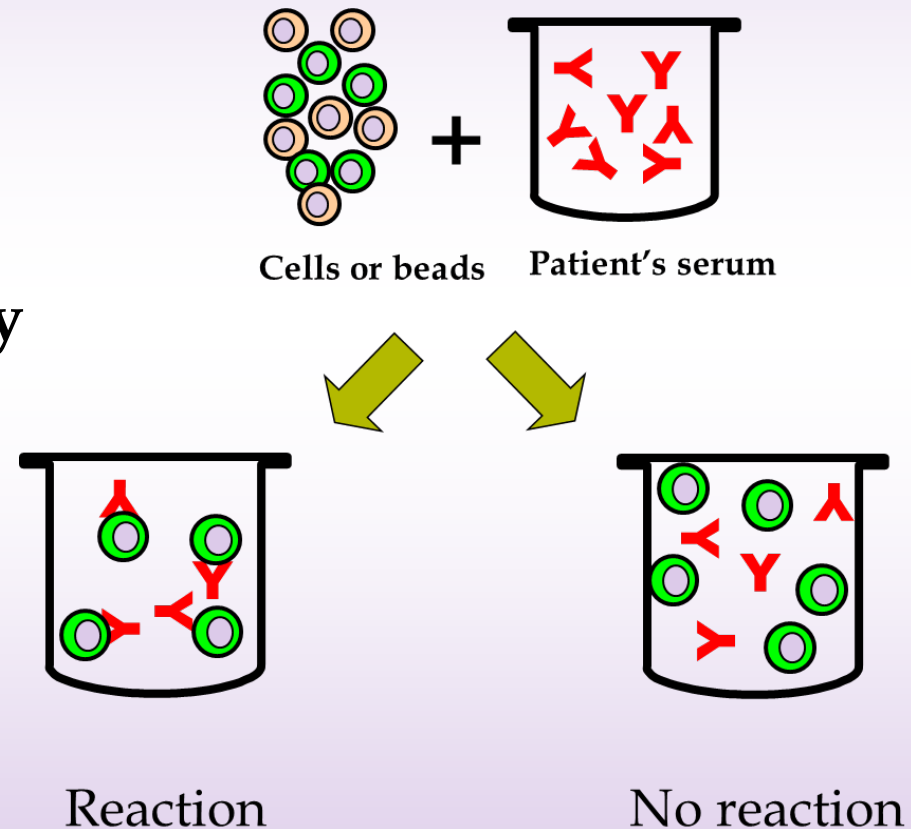
- Serum is the liquid in which blood cells are suspended.
- When a tube of blood is spun in a centrifuge the serum and red cells separate.
- Antibodies are found in serum

Tube of freshly-drawn whole blood



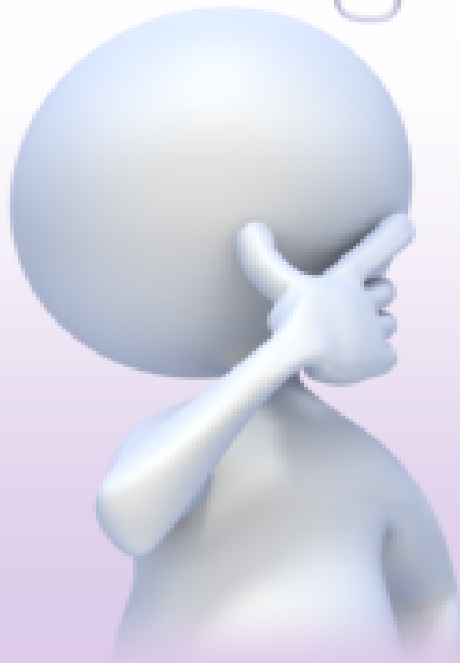
How do you test for antibodies?

- The test for HLA antibodies is called "PRA"
- PRA = Panel Reactive Antibody
- The test mixes cells from a donor with a known HLA type or plastic beads with a certain HLA on them with serum from a patient.



HLA Antibody Detection

- Three questions we ask in antibody detection.

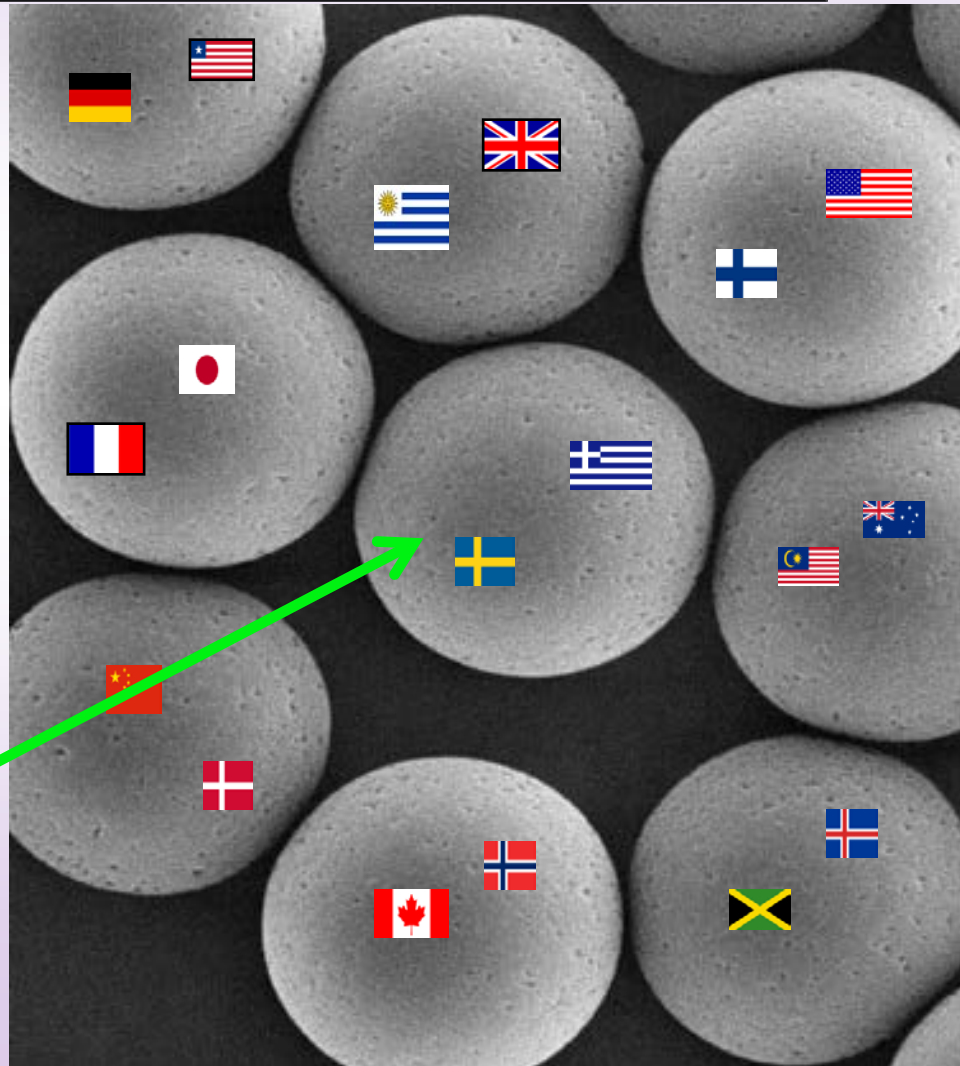


1. Is there an anti-HLA antibody present?
2. Is the antibody clinically relevant?
3. Can we identify acceptable or "safe" antigens that a patient is NOT reacting to?

Back to flags!

- Here are some “cells” with their different HLA flags on them.
- Let’s say that a patient has an antibody to the color red.
- Which one of these cells would not react with the patient’s antibody to red?

This one



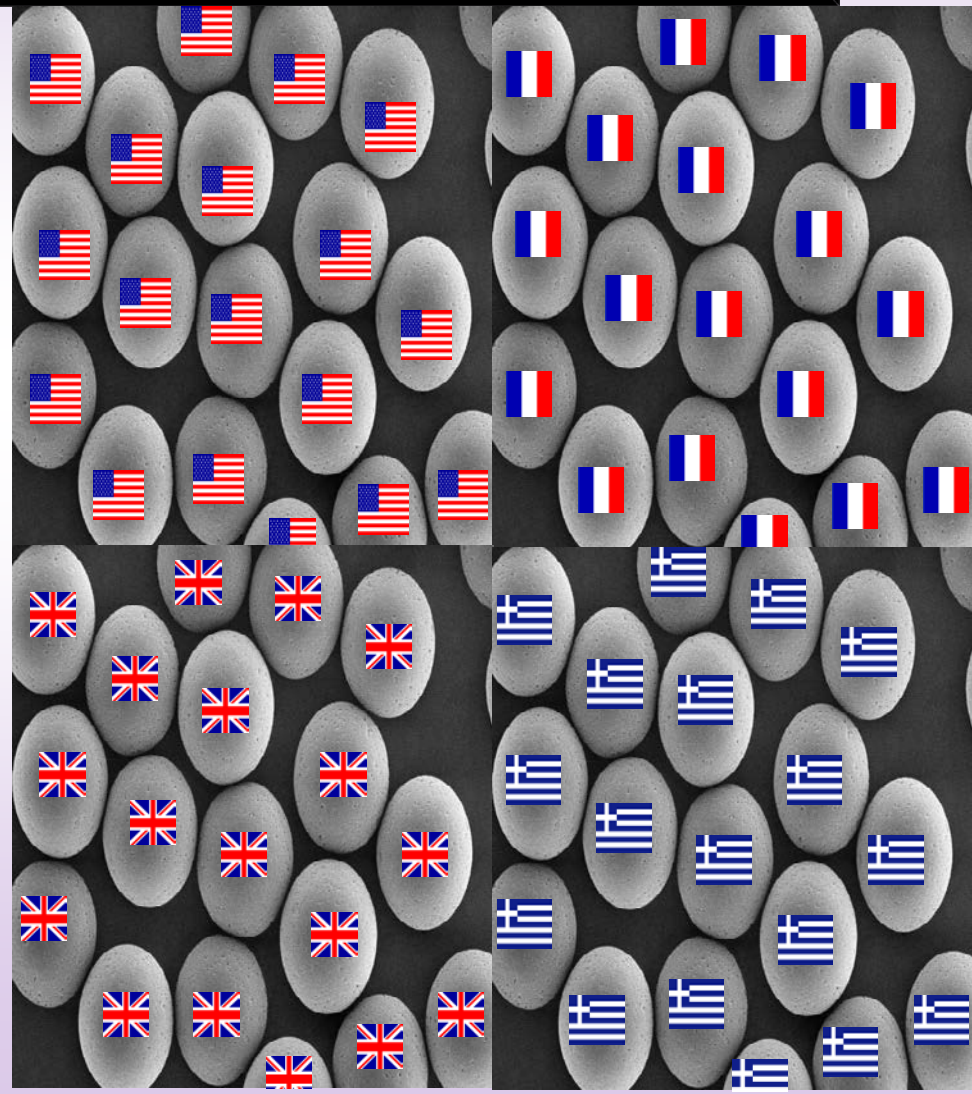
So what?

- Well, this means that if a patient has an antibody to the color red, then they should be compatible with a donor whose flags do not have red in them.
- That's one of the ways we decide who gets the opportunity to receive a transplant.



Additional Tests

- There is another test.
- HLA flags are put on beads by themselves
- This allows us to determine if a patient is truly reacting to one flag by itself.
- This lets us determine if there are any flags that can be considered safe for the recipient.

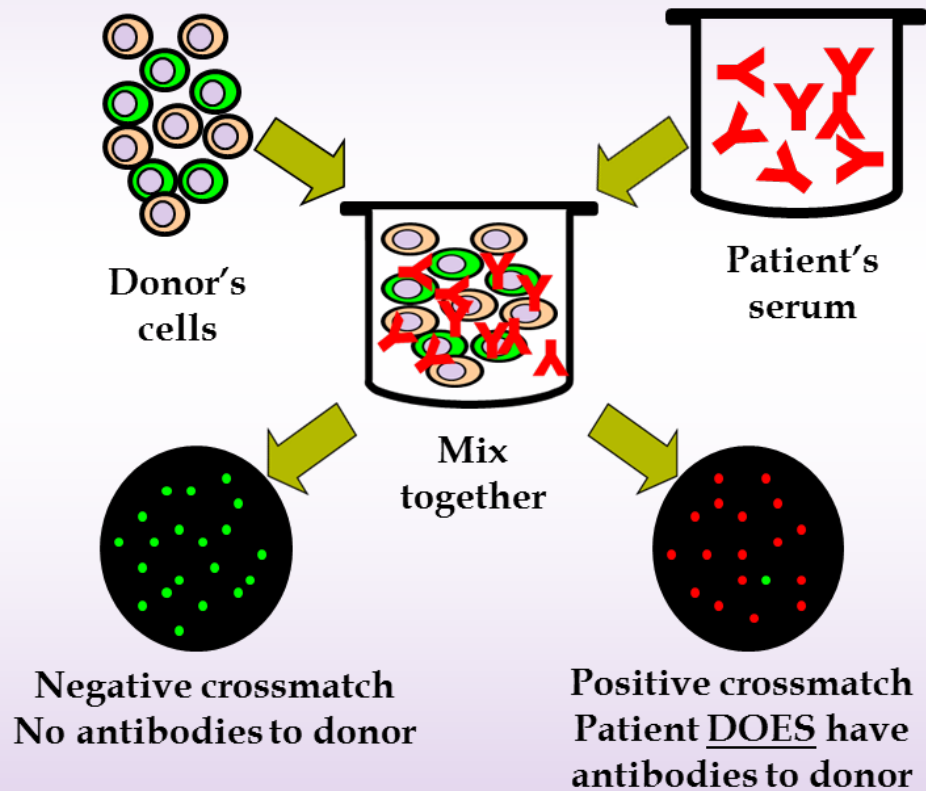


Antibodies Continued

- **As you can probably tell, the PRA test is very important!**
- **The PRA lets us know which HLA antigens (flags) in a particular donor need to be avoided.**
- **The ultimate test to determine if a donor is compatible with a patient is called a crossmatch.**

What is a Crossmatch?

- The crossmatch is a test to determine if a patient has antibodies to a particular donor.
- The crossmatch is performed by mixing the patient's serum with the donor's cells.
- If the patient has antibodies, the crossmatch is positive. This is not good.



Let's Review

- **HLA antigens (flags) are the way that the immune system finds out if something is self or foreign.**
- **The amount of difference between a donor's flags and the recipient's flags can really make a difference in whether or not a transplanted organ survives.**
- **Antibodies to a donor's flags can do considerable damage to a transplanted organ.**

What do HLA Labs do?

- We basically do two things.
- We determine degree of mismatch between donor and recipient
 - HLA typing
- We identify clinically relevant anti-donor HLA antibodies
 - crossmatch (determines donor/recipient compatibility)
 - serum screening (ongoing evaluation of patient's immune status)

Thanks!

- We really appreciate your interest in HLA labs and the important job we do in ensuring that organ and bone marrow transplantation happens in a safe and expeditious manner.
- If you have additional questions please check out the following sites.
- www.ashi-hla.org
- www.donor.gov
- bethematch.org

Thanks!

THANK YOU

Merci

Gracias

Hvala

Obrigado!

Ευχαριστώ

תודה

شكراً

ขอบคุณ

Vielen
Dank

Díky

Grazie

Bedankt