Welcome to the Antibodies Detection and Identification sponsored by the Professional Acknowledgement for Continuing Education, P.A.C.E.® accredited program.

In 1977, P.A.C.E.® was established by the American Society of Clinical Laboratory Science (ASCLS) to stimulate and serve as the quality assurance mechanism for continuing education programs offered to clinical laboratory professionals. ASCLS strongly supports continuing education opportunities to increase and enhance professional knowledge and ongoing proficiency. P.A.C.E.® ensures that the continuing education programs offered to clinical laboratory professionals maintain high standards of quality and professional acceptability.

Blood Systems is an approved P.A.C.E.® Provider and will sponsor continuing education programs.
My name is Marie Holub and I am an Education Coordinator for Blood Systems.

This program is intended for personnel performing antibody detection and identification. The level of instruction is intermediate and this PACE program has been approved for 1.5 contact hours.

There will be a post test evaluation required for PACE credit.

Before we get started, please make sure you have the following materials available to you: Program Summary, Slides, and Case Study Materials.

It is important that you follow the instructions in the PACE Program Guide to ensure you are given credit for participating in this program. If you have any questions, you may contact the Blood Systems Training and Education Department or myself at the number listed above.
At the completion of this class, participants will be able to:

- Describe the primary and secondary immune response.
- List blood group system characteristics.
- State the requirements for antibody detection.
- Describe antibody detection techniques.

At the end of this program, the participant will be able to:

Describe the primary and secondary immune response. The ability to produce antibody directed against blood group antigens is the reason we perform antibody detection testing.

List blood group system characteristics. These characteristics aid in the identification of the antibody and the determination of clinical significance.

State the requirements for antibody detection. These are the requirements of regulating agencies.

Describe antibody detection techniques. As you are aware, there are several techniques available to perform antibody detection and identification.
Demonstrate the process of including and excluding antibody specificities when performing antibody identification. It is as important to exclude clinically significant antibodies as it is to identify the antibody present correctly.

Assess antibody significance to make transfusion recommendations. After determining the antibody or antibodies present, we need to make a recommendation for transfusion. This recommendation should result in adequate survival of the transfused red cells.

The decision to transfuse and the responsibility for the transfusion outcome belongs to the patient’s physician. Our responsibility is to provide the patient and physician with the best red cell component possible.
Blood group antibodies are produced by B cells in response to stimulation by a foreign antigen. This foreign antigen can be a substance in nature or blood group antigen.

The foreign antigen is recognized by phagocytic cells such as macrophages, monocytes or dendrite cells. These cells are known as antigen processing cells. The antigen is processed and then displayed on the surface of the cell by a major histocompatibility molecule.

Helper T cells then recognize the antigen epitope, become activated and then interact with a B cell to initiate the production of antibodies specific for this antigen epitope.

B cells are located in the lymph nodes which explains why our lymph nodes swell when we get sick. Our B cells are producing antibody against the invading organism. This is a simplistic explanation of a very complex process.

Image taken from internet
www.healthsystem.virginia.edu
After the antigen is presented to B cells, some transform into plasma cells producing antibody and some become memory cells.

The primary immune response takes 5-10 days to produce detectable antibody. The sensitivity of our test systems may not detect these antibodies for weeks. This explains why patients that are transfused for the first time rarely exhibit red cell destruction during their hospital stay. The patient may or may not experience symptoms if red cell destruction occurs weeks after the transfusion or may not recognize that what they are experiencing is due to red cell destruction.

If there is no further stimulation, the immune system has a mechanism to turn off antibody production and the antibody level will decrease.

The first immunoglobulin produced is IgM. As you remember this immunoglobulin is a pentamer with 5 subunits held together with a J chain. The pictures are representations of the immunoglobulin structure. Refer to the bibliography at the end of this presentation for references discussing the size and structure of immunoglobulins.

IgG immunoglobulin is produced a little later in the immune response.

The heavy chain of the immunoglobulin molecules have domain areas capable of activating the complement cascade and being recognized by phagocytic cells in the reticuloendothelial system. These two mechanisms are responsible for red cell destruction. Complement activation can lead to intravascular red cell destruction. The domain being recognized by macrophages results in extravascular red cell destruction.
The second exposure to the antigen results in an anamnestic immune response. The memory B cells can begin antibody production much faster and detectable antibody is usually present in 1 to 3 days. A much larger amount of IgG immunoglobulin is produced and red cell destruction can occur.

Depending upon the antibody produced and ability of the antibody to bind complement, red cell destruction can be either intravascular or extravascular.

The image on this slide depicts spherocytes which are found when extravascular red cell destruction occurs. Because of the antibody attached to the membrane, phagocytic cells remove some of the membrane making the cell smaller and rounder than a normal red cell.

I have given you an overview of the immune response. Obviously, this biological function is very complex. For more information on the immune response you can refer to references in the bibliography.

Now, let’s discuss the blood group antigens and antibodies.

Image taken from Internet
www.hoslink.com
There are five classes of immunoglobulins. IgA immunoglobulins are secretory and present in mucosal secretions. IgA is thought to combine with environmental antigens forming antigen-antibody complexes that are eliminated as surface secretions are excreted. IgD exists as membrane immunoglobulin on unstimulated B cells. The function of this immunoglobulin is unknown. You may be familiar with IgE immunoglobulins if you suffer from allergies.

As previously mentioned, IgG and IgM immunoglobulins are of importance in immunohematology. These are the immunoglobulins produced in response to blood group antigen stimulation.

All immunoglobulins are composed of 2 heavy chains and two light chains. The heavy chain determines the immunoglobulin class. The light chains and heavy chains are held together by disulfide bonds. The Fab part or area where the light and heavy chains are located provide the antigen binding capacity. The Fc or heavy chain only area contains the domains for complement activation and receptors for macrophage binding.

The Fc part of the molecule is important in destruction of red cells. It is this part of the antibody molecule attached to a red cell that will activate the complement cascade leading to intravascular hemolysis or cause a macrophage to attack a sensitized red cell.
Blood group antigens were named as the corresponding antibody was discovered. Many times the antibody/antigen was named for the patient the antibody was detected in or for the person who discovered the antibody. Therefore, there was no consistency in naming the blood group system antigens and antibodies.

Several antigens may be grouped into one system depending on the genetics of the blood group system. Since there is usually more than one antigen in a system, we must have a way to record the blood group system phenotype. The phenotype is the antigen observed on the red cell and may not be the genotype. In an effort to standardize the blood group system nomenclature, the ISBT has assigned numbers to each blood group system and antigen. The ISBT is the International Society for Blood Transfusion.

The following slides will list the major blood group systems and their nomenclature or terminology.
The ABO system is not usually considered when talking about antibody detection and identification; however, I have included the ABO system because it is the most important blood group system.

This system can also be used to demonstrate the difference between phenotype and genotype. The left column of the slide lists the antigens, phenotype and ISBT number and the right hand column lists the genotype. You will notice that a group A person can have two chromosomes coding for the A gene or one chromosome coding for the A gene and one for the O gene. The O phenotype reflects the absence of the A and B antigens. I bring up the genotype to use it as a reference later when we discuss homozygous and heterozygous terminology. I will not discuss genetics of other blood group systems unless it is pertinent to the understanding of a blood group.

The ISBT number is not used routinely in everyday operations and communication. This number can be converted to a barcode on the donor unit label and will be recognized anywhere in the world that the unit is distributed because it is the universal standard. This this the ISBT 128 labeling symbology that should have been implemented on May 1, 2008 with the effective date of the 25th edition of the Standard for Blood Bank and Transfusion Services published by the AABB.

For today, I will not include ISBT numbers for the rest of the blood group systems being discussed. Image from Internet ghr.nlm.nih.gov
The blood group systems included are those whose antibodies are most often encountered in a transfusion service. Each system has more antigens than I have listed on the slide; however, the corresponding antibodies are not likely to be present or recognized.

The RH system is second in importance to the ABO system. Anti-D and anti-E are the most common Rh antibodies detected in pre-transfusion testing. The Rh antigens are located in the RBC membrane.

The MNS system antigens are located on the sialoglycophorin chains that extend above the RBC membrane. Anti-M and -N are usually naturally occurring antibodies while anti-S and -s are immune in nature. I will discuss the difference between naturally occurring and immune antibodies in the antibody immunoglobulin class discussion.

The Lewis system is unique in that the antigens are not integral parts of the RBC membrane. These are plasma antigens that absorb onto the surface of the RBC.

The P system is associated with naturally occurring antibodies. The P1 antigen is an antigen that appears in a soluble form and can be found in pigeon eggs and droppings.

Refer to the bibliography for references if you wish to learn more about each system.
Anti-K is another antibody that is frequently encountered in a transfusion service. In Kell system, the K, Kpa and Jsa antigens are of low incidence and k, Kpb and Jsb antigens are of high incidence. Incidence refers to the frequency of the antigen in the population. Low incidence means the antigen is not common or a low % of the population have the antigen. High incidence means that most of the population has the antigen and an individual lacking the antigen is rare.

The Duffy system has an antigen, Fy3, associated with a particular ethnic group which is the African Americans. This is an example of genetic selection. The Fya and Fyb antigens serve as receptors for a particular Malarial parasite, *Plasmodium vivax*. Individuals lacking these antigens are not susceptible to this form of malarial infection. The result is that about 68% of the African American population lack the Fya and Fyb antigens and are also Fy3 negative. The significance is that many patients with sickle cell disease are Fy3 negative making it very difficult to obtain units for transfusion when anti-Fy3 is present.

The Kidd blood group system antibodies are very dangerous. Besides ABO antibodies, these antibodies are one of the few that can cause intravascular hemolysis. The danger lies in the immune response to these antigens. The antibody level drops off rapidly after stimulation and is not detectable the next time antibody detection is performed. Anti-Jka is responsible for many delayed hemolytic transfusion reactions.
The clinical significance of an antibody depends on whether it causes red cell destruction or not. Red cell destruction depends upon the immunoglobulin class, ability to activate complement and macrophage receptors.

This ability to destroy red cells can be observed first hand or has been observed and reported in the literature. Providing antigen negative units for transfusion depends upon the clinical significance of the antibody. If the antibody causes red cell destruction, known antigen negative units are supplied for transfusion. If the antibody does not cause red cell destruction, crossmatch compatible units are suitable for transfusion regardless of the antigen status.

Knowing the blood group system characteristics and clinical significance is important in providing red cell components for transfusion.

If an antibody has been reported to cause hemolytic transfusion reactions and/or hemolytic disease of the fetus and newborn, known antigen negative units are required for transfusion.
Like the P1 antigen found in pigeon eggs, there are other substances in nature that look like red cell antigens. When an antibody directed against a red cell antigen is detected in a person who has never been transfused or pregnant, we consider this a naturally occurring antibody and it is usually IgM in nature.

Blood group system antibodies detected after transfusion or pregnancy are considered to be produced in response to immune stimulation and are IgG in nature.

You are probably wondering why we consider the production of IgM antibody naturally occurring, when there must have been an immune stimulation to produce the antibody. What is actually being differentiated is whether the antibody production is stimulated by red cells or a substance in nature.

This chart lists the blood group system antibodies that are primarily IgM or IgG in nature. Just remember that the antibodies do not read the books and there are always exceptions to the rule.
As previously discussed, the ability to activate complement is significant in determining the antibody’s clinical significance.

The complement cascade is composed of 9 molecules. If complement components 1-4 are activated and the cascade stops at that point, red cell destruction, if it occurs, is extravascular.

If complement activation proceeds through all 9 components, red cells are destroyed intravascularly. The blood group systems known for intravascular destruction include the ABO and Kidd systems.

IgM molecules are usually better at activating complement because they consist of 5 subunits. The IgM immunoglobulin has Fc portions of the molecules in close proximity. In contrast, 2 IgG molecules must be present on the red cell fairly close together to activate complement. The location of the antigen on the red cell membrane and the number of IgG molecules on the cell will impact the ability to activate complement.
This slide lists the blood group systems associated with hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. You will note that the blood group systems that are predominantly IgM do not appear on these lists. As you recall, IgM antibodies cannot cross the placental barrier. IgG molecules do cross the placenta and attach to fetal red cells with the corresponding antigen.

The Rh system causes the most severe form of HDFN which may result in intrauterine death. It is critical to determine the clinical significance of any antibody detected in a prenatal patient. Our responsibility is to provide this information to the patient’s physician. The physician will decide on how frequently to monitor the fetus if HDFN can occur.

Decreased red cell survival occurs most often with IgG antibodies. Once again I want to remind you that even though the blood group systems with predominantly IgM antibodies are not thought to be clinically significant, many have been reported with an IgG component or for whatever reason are IgG in nature. In these instances, known antigen negative units would be appropriate.

Now that we have discussed the blood group systems and their antibodies, let’s talk about antibody detection.
Standard 5.8.3.1 refers to antibody detection on donor units. It is impractical to separate donors that have been pregnant or transfused from those that have not in testing labs. Therefore, every donation is tested for unexpected antibodies. If an unexpected antibody is present, the Code of Federal Regulations requires that the donor unit be labeled with the antibody specificity if the unit is labeled for distribution.

Standard 5.13.3 refers to patient testing. A sample must be drawn from the patient and tested for unexpected antibodies. If the patient has been transfused or pregnant in the preceding 3 months, a sample must be obtained within 3 days of the scheduled transfusion.

We all know how easy it is to get accurate transfusion and pregnancy history on the patients for which we perform pretransfusion testing. It is easier and maybe safer to apply the three day rule to all patients requiring pretransfusion testing.
Many donor testing labs use automated non-tube testing techniques that upload test results to laboratory information systems. These labs may use a pooled antibody detection reagent consisting of one vial of pooled red cells.

Hospital and blood center transfusion services are also moving toward non-tube testing techniques that may be manual, semi-automated or automated. The requirement for patient antibody detection is the use of a non-pooled antibody detection reagent consisting of 2 or 3 vials of a single reagent red cell. An autocontrol is not required when performing antibody detection.

The advantages of non-tube testing techniques include the ability to batch testing which increases productivity and eliminates the subjectivity of shaking test tubes and grading reactions. The decision to use non-tube testing techniques depends on volume of testing, workflow requirements, staffing and last but not least, budget.

Tube testing techniques offer the advantage of different phases of testing including immediate spin, a 37C reading and the antiglobulin phase of testing. Different blood group system antibodies react at different phases of testing giving clues to the specificity of the antibody.

We will discuss the significance of the type of reactions seen at different phases of testing as the presentation progresses.

Do not become overwhelmed with excitement when an antibody is detected.
There are clues to the identity of the antibody or antibodies present in the test results that we observe.

Non-tube testing techniques include only one phase of testing - the antiglobulin phase. However, you may see variation in the strength of reactions.

Reactions seen at different phases in tube testing can point to a specific blood group antibody. Reactions seen at immediate spin are usually caused by IgM antibodies. Reactions at the antiglobulin phase are due to IgG antibodies. Reactions observed after a 37°C incubation may be IgM antibody reactions carrying over or an IgG antibody. It is important to know the characteristics of the antibodies.

The pattern of reactivity determines the specificity of the antibody present. If only a few reagent red cells are positive, then look for an antibody whose corresponding antigen has a low incidence such as anti-E or anti-K. If almost all cells are reactive, the antigen will be more frequent and you would look at blood group systems whose antigens have a higher incidence.

The autocontrol, again, is not required in antibody detection, but it must be included in antibody identification. This will tell you whether the antibody you have detected is an auto or alloantibody. This information will have significance in making a transfusion recommendation.
Antigen variability is a characteristic of the P1 antigen. The number of antigen sites or copies varies from person to person. The strength of reactivity may vary from 2+ to negative depending on the number of antigen sites on the reagent red cells.

Dosage is another reason reaction strengths may vary. Dosage is determined by the genetic inheritance of the antigens. Different blood group genes in the same system are considered alleles. For example; the genes for big C and little c are alleles, M and N are alleles and Jka and Jkb are alleles. This means that if two big C genes are present and no little c genes are present, the individual is considered homozygous and there are more copies of the antigen on the red cell. A person with a big C gene and a little c gene is heterozygous and will have fewer copies of the big C antigen on their red cells. A homozygous expression of a gene will have a double dose of antigen sites and the heterozygous expression of a gene will have a single dose of antigen sites.

Dosage is when a antibody reacts stronger with a double dose expression of antigen than with a single dose expression.

Multiple antibodies present may also demonstrate different strengths of reactivity with the reagent red cells.

This image represents variation in reaction strength. If you look closely, you can see effacement of red cells in some wells. The test method is the Solid Phase Red Cell Adherence assay from Immucor.
IgM and IgG antibodies react best at different temperatures.
IgM antibodies react best at immediate spin and 4C and the molecules are large enough to agglutinate without the addition of any other reagent.
IgG antibodies react best at 37C before adding the antiglobulin reagent. IgG molecules are too small to cause agglutination by themselves. A red cell sensitized with IgG molecules can not be detected without the addition of an antiglobulin reagent. Remember that once test tubes have been removed from 37C incubation, the cells must be washed and antiglobulin reagent added without delay. Delay can cause the IgG molecule to detach from the red cell giving a false negative reaction.
Reactions at IS would indicate a blood group system antibody that is usually IgM in nature such as anti-M, -Leb or -P1.
Reactions at all phases are characteristic of anti-K and -Lea.
Reactions at 37C and the antiglobulin phase is characteristic of the Rh antibodies.
Reactions only at the antiglobulin phase is characteristic of anti-Jka or -Fya.
This image depicts the indirect antiglobulin test in which IgG antibodies are attached to red cells in vitro and an antiglobulin reagent is added to detect the sensitized red cells.
Image from Internet www.merck.com
The pattern of reactivity is matched to the pattern of positive and negative reactions on the antigen profile sheet or panel sheet. I will go over this process in detail.

The number of positive and negative reactions is important in determining the probability that the observed pattern is not due to chance alone. The standard approach used is to have three antigen positive cells that react and three antigen negative cells that are non-reactive. This may not always be possible when multiple antibodies are present, but should at least be used whenever possible.
The autocontrol is very important in antibody identification. The presence of a clinically significant alloantibody requires us to find antigen negative units for transfusion.

If an autoantibody demonstrates a recognizable specificity, antigen negative units may not be recommended. In this instance, transfusion of antigen negative units usually does not guarantee better survival than transfusion of antigen positive units.

It is important to reserve antigen negative units, especially those that are more difficult to find, for patients with alloantibodies.

Now let us look at antibody detection and identification results.
You receive a type and cross order for 4 units on a patient going for a total hip procedure today. The antibody detection test is positive and when you call to get more information, you are told the patient is in surgery already.

Does this sound familiar? I am sure that we have all experienced this one time or another.

We usually do not have any patient history available and may not have any previous history to help us out.

The antibody detection test and crossmatch were performed at the same time to minimize delay in supplying units if needed.

Regardless of the technique used to perform antibody detection, strict adherence to the procedure and manufacturer’s instructions is critical to the quality of test result obtained. Each different technique is designed to achieve the optimal conditions for antibody detection.

Let’s look at the initial test results.
The interpretation of the ABO/D type is A, D positive.

This antibody detection was performed using a tube technique. The additive used must have been a low ionic strength solution or LISS reagent since we have reactions at the 37C and antiglobulin test or AGT phases. If the additive was polyethylene glycol or PEG, reactions would have been seen at AGT only.

The IS phase is negative with all three cells. The 37C phase has a 2+ reaction with cell number 2 and all cells are reactive at AGT. The clues we have are the different phases of reactivity and the different strengths of reactions. These clues point us toward a possible Rh antibody because of the reactions at 37C and another IgG antibody reacting at the AGT phase only, such as an antibody in the Duffy or Kidd blood group systems. I would not expect an antibody from a blood group system with IgM antibodies because there are no reactions at the IS phase.

The next step is to perform an antibody identification. We will use a tube technique with a LISS reagent.
You can refer to the handout Case #1 Antibody Identification provided or to the panel on the screen. In looking at the phases of testing, you can see that the panel reflects the same type of reactions seen in antibody detection. All cells are nonreactive at the IS phase, there are two reactions at the 37C phase and there are different strength reactions at the AGT phase. There are positive and negative cells at the AGT phase. The check mark next to the negative reaction at the AGT phase indicate the antiglobulin control cells were positive and the negative results are valid. The autocontrol is negative telling us the antibodies present are alloantibodies. Now we are going to look at the pattern of reactivity.

Immediate spin does not contribute any information to specificity. However, at 37C we have 2 cells that are reactive - cells 3 and 6. The next step is to look at each column to see if there is a pattern match. We will start with the D column. If anti-D was present, we would expect to see positive reactions with cells 1, 2, 3, 4 and 11. This does not match our pattern of cells 3 and 6. The C column has positive indicated on cells 1, 2 and 5 which also does not match. The E column has positive indicated on cells 3 and 6. This does match the pattern seen at 37C. From this we can determine that the antibody reacting at 37C appears to be anti-E. Let’s look at the pattern at the AGT phase.
The positive reactions are found on cell number 2, 3, 6, 8, 9 and 11. At this phase of testing we do have at least 3 cells that are reactive and 3 cells that are nonreactive. Looking at this pattern, what antibody or antibodies do you feel are present at the antiglobulin phase. Locate the pause button identified by two colored vertical lines. They will be located in the media task bar. Click on the pause button when you are ready to compare the pattern of reactivity. Restart the recording when you have finished.

The pattern of reactivity matches that of the Fya column. How do you explain the different strengths of reactivity? Cell number 3 and 6 are 4+, cells 2 and 11 are 2+ and cells 8 and 9 are 1+. The 4+ reactions on cells 3 and 6 appear to be due to anti-E. When you look at cell number 2 and 11 in respect to the Duffy antigens, you will note that these two cells are double dose cells. This means that the cell donor is apparently homozygous and has two Fya genes coding for the Fya antigen. Cells 8 and 9 are single dose cells. Both the Fya and Fyb antigens are present on these cells with a single dose expression of the antigen. Remember dosage is an antibody reacting stronger with a double dose expression than a single dose expression of the antigen. The reactions seen with anti-Fya is an example of dosage.

The blood group systems in which their antibodies show dosage are listed on the next slide.
The antibodies to the P1 and Lewis antigens do not appear on this list because they do not have antithetical antigens. Do keep in mind that you can see different strengths of reactivity due to antigen variability.

The Technical Manual recommends that we establish an antibody identity by using 3 antigen positive cells that are reactive and 3 antigen negative cells that are non-reactive. We may not be able to meet this standard if multiple antibodies are present. A more liberal approach is to use 2 positive cells and 3 negative cells. This is derived from calculations by Harris and Hockman. Please refer to the Technical Manual for a more detailed discussion of probability.

Now that we have agreed that the antibodies present are anti-E and -Fya, have we met the criteria for antibody rule in and rule out? Let’s look at the panel again.
The two E cells are also Fya positive. Can we use these cells to confirm the anti-E specificity because they are positive for both antigens? In most cases, you would consider the reagent red cell only if one antigen is on the red cell when multiple antibodies appear to be present. So we would not use only these cells to rule in or include the antibody. The supporting clues are the reactions at 37C which are only on the E positive cells. Do we meet the criteria of 3 antigen positive cells that are reactive and 3 negative cells that are nonreactive? We have 2 E positive cells reacting at 37C and 4 Fya positive cells that are E negative. There are 5 negative cells. Remember you can use the reactions seen with the antibody detection cells to complete your rule and rule out. Using the antibody detection cells, we do have 3 E positive cells that are reactive at 37C. You would need to follow the policy of your facility to rule in anti-E using 37C phase of testing. If your requirement is to rule in antibodies on only cells that are positive for one antigen, additional testing would be required.

The next question is are all other clinically significant antibodies ruled out or eliminated? In order to perform antibody rule out, only cells nonreactive at all phases of testing are acceptable. The technique to perform rule out is to take the first nonreactive cell, which is cell number 1 and look at the antigens present and absent on the cell.
Looking at only the cells that are nonreactive, cell number one has the D, C, e, M, N, s, P1, Leb, Fyb, Jka and Jkb antigens present. I have drawn a black line through the box listing the antigen when the antigen is present. We cannot use cells 2 and 3 for rule out because they are reactive. The next cell we can use is number 4.

I want you to also notice that the antigens highlighted in yellow represent double dose antigens, the light green represents single dose antigens and the pink represent antigens that have no antithetical antigen but do exhibit antigen variability.
The E, Fya and Lea columns do not have any lines. We can say that the patient has anti-E and -Fya. Do we need to worry that we have not eliminated anti-Lea? Review the policies and procedures at your institution to determine if anti-Lea must be ruled out. This antibody is not considered clinically significant; therefore, many facilities do not attempt to eliminate anti-Lea. If your policies require you to rule out anti-Lea, then make sure you do.

You will note that the S and K columns have only one line or hash mark. There is no requirement to rule out clinically significant antibodies with more than one cell. There does not appear to be any consensus regarding rule criteria. It is important to keep in mind the blood group systems that show dosage and attempt to eliminate antibodies to these antigens with double dose cells.

Another technique that can be used to confirm or eliminate antibodies is the use of ficin pretreated red cells. The reactivity of some blood group system antibodies is enhanced with the use of ficin and some are destroyed.

The decision was made to perform a ficin panel and the results follow on the next slide.
It is required that the cells used for ficin pretreatment are the same cells tested in the initial antibody identification. This panel has been modified to remove the IS and 37C columns and the results of the ficin panel have been added in the last column. The pattern of reactivity with the ficin pretreated cells is different from the initial panel. You will notice that some reactivity has been destroyed. Cell number 3 and 6 are still positive while cells 2, 8, 9 and 11 are now nonreactive. It is also important to note that an autocontrol was included in ficin pretreatment of the red cells. An autocontrol should be tested with each different technique.

Let’s review the blood group antibody characteristics with ficin pretreatment of the reagent red cells.
Antibody-antigen reactions are enhanced when using ficin pretreated red cells with the Rh, P1, Lewis and Kidd antibodies. Reactions are destroyed with Anti-M, -N, -S, -Fya and -Fyb. This supports the requirement that a ficin pretreated panel should never be performed by itself with no untreated cell panel for comparison. If the patient has anti-Fya or -S, you would never know since the reactions would be destroyed. The reactions seen with the ficin pretreated red cells supports our conclusion that anti-E and -Fya are present in the patient’s plasma.

We can also use the ficin panel to rule out other blood group system antibodies that are not destroyed by ficin. We can rule out the presence of anti-K because the K positive cells 2, 9 and 10 are nonreactive and the K antigen is not destroyed by ficin pretreatment. However, we cannot rule out anti-S because the S antigen is destroyed by ficin pretreatment.

Another tool available is the use of a selected cell panel to complete the rule out process. Reagent red cells can be selected from other panels and tested. The cells selected should be negative for the antigens to the suspected antibody or antibodies and positive for the antigens needed to eliminate additional antibodies.

I will discuss this in detail with Case #2.

The final step in antibody identification is to perform antigen typing to confirm that the patient lacks the antigen to the corresponding antibody identified. This relates back to the immune response in that we form antibodies to foreign antigens.

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<th>Reactions Enhanced</th>
<th>Reactions Destroyed</th>
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<tr>
<td>RH</td>
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Antibody Detection and Identification

**Ficin Effects**
A 63 year old female was admitted to the emergency room with a fractured pelvis. The patient is now on her way to surgery.

Those of us performing pretransfusion testing for facilities with emergency rooms encounter this situation or one like it almost every day.

The testing performed for this patient used non-tube testing techniques. Let’s review the initial testing results.
The ABO/D typing interpretation is B, D negative. The antibody detection test is positive with all three cells tested. There is some variation in reaction strength. Let’s look at the results of the antibody identification panel. This is a non-tube testing technique and only the antiglobulin phase is tested. According to the Standards, there is a 37°C incubation before the antiglobulin test is performed.
In looking at this panel, we note that there are positive and negative reactions and the autocontrol is negative. There are no checkmarks at the AGT phase for antiglobulin control cells because the non-tube testing techniques do not use them. The antibodies present are alloantibodies. Notice I said antibodies. I said this because of the different strengths of reactions. Since the patient is D negative, the first antibody I look for is anti-D because this is one of the antibodies most frequently encountered in a transfusion service. The D antigen is present on cell number 1, 2, 3, 4 and 11. Looking at the testing results, there are positive reactions on cell number 1, 2, 3, 4, and 11 supporting the suspected presence of anti-D. In addition, cell number 9 and 10 are also positive.

The next thing I will do is check to see if the cells with the 3+ reactions fit a pattern. I want you to look at this pattern to determine if it matches a recognizable pattern in the panel antigen profile.

Locate the pause button on the media task bar and pause the recording to review the 3+ reactions. Restart the recording when you have finished.

The 3+ reactions match the pattern for K. I would suspect that the antibodies present are anti-D and -K. The next step is to verify that all additional clinically significant antibodies have been eliminated. Pause the recording to perform antibody rule out if you have not already done so.

Let’s look at the results of antibody rule out.
There are no hash marks in the D, Lea and K columns. We have already decided that the antibodies present are anti-D and -K. You need to follow your policies and procedures for ruling out anti-Lea. The C, E, S and P1 columns have only one hash mark. We have not met the rule out criteria for anti-C, -E and -S. You notice that I have only one hash mark for the P1 column and there are 2 nonreactive cells that are P1 positive. I would not use cell number 8 for rule out because the antigen profile indicates this cell has a weak expression of the P1 antigen. We always want to use the strongest expression of the antigen to rule out antibodies. In a perfect world, we would use only double dose antigen positive red cells for rule out. As you noted, the 4 cells used for rule out included double and single dose antigen positive cells.

Since the use of a ficin panel is not useful because the reactions with both suspected antibodies are not destroyed, a selected cell panel would be required to complete the rule out of anti-C, -E and -S. Again, anti-P1 is not considered clinically significant and there is no evidence to suspect the presence of anti-P1. Follow the requirements in your laboratory for ruling out the presence of clinically insignificant antibodies.

The selected cell panel has been chosen and we will review the red cell selection and results.
You will note that all selected red cells are D negative, but I have included a K positive cell. I do recommend including an expected positive cell as a positive control. This ensures that the correct patient’s serum or plasma has been added and the test has been performed correctly. I chose a K pos cell because I had 3 D positive cells that are K negative, but only 2 K pos cells that are D negative. We need to test additional C, E and S positive cells that are D and K negative. It is almost impossible to find double dose C and E cells that are D negative because those phenotypes are very rare. Therefore, we have to complete rule out with single dose cells. I did try to select S positive cells that are double dose and in doing so, also got additional P1 positive cells and double dose cells for the Duffy and Kidd antibodies. The reason I am emphasizing this point is that you need to keep in mind those blood group systems that show dosage. Whenever possible, rule out should be performed with the strongest expression of the antigen. Remember in Case #1, we had an example of an antibody showing dosage. There are times when an antibody may react weakly with a double dose cell and not at all with a single dose cell. If you use only single dose cells for rule out in this situation, you will eliminate a clinically significant antibody. You can not depend upon the crossmatch to detect the corresponding antigen because the donor unit may be single dose also.

This selected cell panel confirms that there are no additional clinically significant antibodies present. Again the final confirmation is antigen typing the patient red cells to confirm that they are antigen negative.

Let’s proceed to the final case study.
You are performing routine testing for a patient scheduled for cardiac bypass surgery. The ABO/D typing is performed in the test tube and non-tube testing antibody detection test is performed.

Let’s look at the initial testing results.
The ABO/D type is O, D positive.
The antibody detection test is positive with all 3 cells and the strength of reactivity is the same on each cell.
Let’s review the antibody identification results.
All cells are reactive and the autocontrol is positive. These reactions are consistent with an autoantibody. Since there is no history of previous transfusions, I would not expect that there would be clinically significant antibodies present. This patient is male so we do not need to consider pregnancy as an immune stimulation. Even if the patient was female, most of the time pregnancy is not a good form of stimulation for red cell antibody production. If this were the case, just about every woman who has been pregnant would have unexpected antibodies and we do not see this in pretransfusion testing.

The next step would be to perform a direct antiglobulin test or DAT and additional serum or plasma testing.
The DAT is positive with IgG only on the red cells. What is the clinical significance of this finding? We will need to evaluate when additional testing such as elution studies need to be performed.

What is the patient’s hemoglobin? Is there any evidence of red cell destruction? If the patient is not anemic and there are no signs of red cell destruction such as an elevated bilirubin, then this serological finding has no clinical significance. Elution studies or any laboratory testing should only be performed when the result aids in the diagnosis or treatment of the patient.

Non-tube testing techniques are very good at picking up autoantibodies. These are antibodies that we don’t want to see. One suggestion is to switch back to a tube testing technique since they are less sensitive and may not detect the autoantibody.

A reminder that the positive DAT will affect any antigen typing that requires an antiglobulin phase and may affect all typings if the red cell is so heavily coated that spontaneous agglutination is seen. Be sure to include an adequate control when performing any typing including the ABO/D typing. There are techniques to remove immunoglobulin from the red cells to facilitate antigen typing, but this is a topic for another discussion.

The decision is to switch to a tube testing technique and the next slide has the testing results.
This testing includes all phases of testing; however, you may choose to perform only the antiglobulin phase. Remember the requirement is to include a 37°C incubation before performing antiglobulin testing.

The antibody detection test is negative. The crossmatch can also be performed using a tube testing technique.

What if the patient had been previously transfused? Would this be acceptable since tube testing is less sensitive than non-tube testing techniques? In my experience, this would be acceptable testing. In order to resolve the serological problem seen with non-tube testing techniques, the patient’s serum or plasma would need to be adsorbed to determine if alloantibodies are present. The technique of choice would be an autoadsorption. This is a time consuming procedure and one that many staff members feel uncomfortable performing.

Would we be missing clinically significant antibodies using tube testing that would have been detected by testing adsorbed serum or plasma with the non-tube testing technique? Again, in my experience the answer is no. There are factors involved in performing adsorption studies that will not guarantee that underlying alloantibodies will be detected using non-tube testing techniques. There will be some dilution of the serum or plasma and the possibility of nonspecific adsorption of an alloantibody, if present, with the autoantibody.

I recommend trying a tube testing technique to determine if it will resolve the serological problem.
We have covered the significance of the immune system in pretransfusion testing. The immunoglobulins produced include IgM and IgG antibodies. I have reviewed the blood group systems including the immunoglobulin class and characteristics such as phase of reactivity, ability to bind complement, effect of ficin pretreatment of reagent red cells and the ability of the antibodies to cause hemolytic transfusion reactions or hemolytic disease of the fetus and newborn.

We have explored the clues obtained from testing observations and how they apply to identifying the suspected antibodies. It is as important to eliminate additional clinically significant antibodies as it is to correctly identify the antibody or antibodies present.

Known antigen negative red cells will be supplied for any antibody identified that can cause red cell destruction. Antibodies that are historically clinically insignificant, supported by the serological observations, do not require known antigen negative units. Crossmatch compatible units have been shown to have expected red cell survival.

I have included a bibliography listing references available if you want to continue your study of blood group systems.
Antibody Detection and Identification

Bibliography

- Standards for Blood Banks and Transfusion Services, American Association of Blood Banks.


Bibliography available.
Follow the instructions here to receive P.A.C.E. credit for completing this class.
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