

Exercise 5

Type and Screen

Textbook: Quinley, Chapter 6 pages 79-85 and Chapter 10 pages 163-166

Skills: 20 points

Objectives:

1. List three situations in which screening for unexpected antibodies should be performed.
2. List two patient populations who are candidates for the Type and Screen procedure instead of the traditional crossmatch.
3. State the advantage of performing a Type and Screen instead of the crossmatch.
4. State the rationale which must be considered when ordering a Type and Screen instead of a crossmatch.
5. State the two testing components involved in performing a Type and Screen.
6. State the reason for performing a second forward typing on each patient who has no history on file.
7. State the principle of the indirect antiglobulin test as it relates to the antibody screen procedure.
8. Define “unexpected antibodies”.
9. State the criteria considered in determining that a blood group antibody is “clinically significant”.
10. List two observations that are considered a positive reaction in blood bank testing.
11. Define “dosage affect” as it relates to blood group antibodies.
12. State the ABO type and source of the antibody screening cells.
13. State the components and purpose of an autocontrol in blood bank testing.
14. State the three phases of testing in the antibody screen and the antibody class detected in each.
15. State the purpose of albumin in the antibody screen procedure.
16. Describe the reagent “Coombs Control Cells” (check cells) and state their purpose for use.
17. State what must be done if Coombs Control Cells do not give the expected reaction.
18. State what must be done if a patient has a positive antibody screen test.
19. List four limitations of the antibody screening procedure.
20. Determine the ABO and D type of a patient red blood cells and detect atypical antibodies present in the patient serum by testing it against screen cells of known antigenic makeup.
21. Perform and interpret the Type and Screen procedure with 100% accuracy.

Discussion

Patients may form antibodies to blood group antigens they do not possess on their red blood cells as a result of exposure to the antigens by prior transfusion and/or pregnancy.

Screening for antibodies in patient or donor serum is an established blood bank procedure. The advantages of systematic screening to detect unexpected antibodies includes: facilitates the selection of “safe” donor blood for transfusion, aids in the prognosis and treatment of hemolytic disease of the newborn, and enables donor blood containing antibodies of potential clinical significance to be recognized.

Before this procedure became popular many units of donor blood were crossmatched and held in reserve for patients who would probably not need it. At times this would cause shortages of the blood supply and unnecessary outdating of donor units. These factors, along with the added expense of crossmatching blood, caused the Type and Screen (T&S) procedure to gain popularity.

*This procedure is most frequently used to screen pre-operative or obstetrical patients whose risk of excessive blood loss is **minimal**. In case of an emergency, where blood is needed for these patients, uncrossmatched ABO and D compatible blood can be released with 99.9% assurance of safety, as long as the patient has no unexpected antibodies. If the antibody screen is positive the patient **is not** a T&S candidate, the antibody present in the serum must be identified and antigen negative donor units must be crossmatched.*

There are hundreds of different antigens on the human red blood cell. So far we have studied the ABO and Rh (D, C,c, E and e) antigens and performed testing to detect the presence or absence of these antigens. The “other” blood group antigens are not very immunogenic but some individuals who have been exposed to these antigens through previous transfusions or pregnancy may be exposed and recognize one or more of these as foreign, resulting in the formation of an antibody which will react with the specific antigen(s) which caused their production.

Screening for the presence of unexpected antibodies in patient or donor serum is an established blood bank procedure. The antibody screen, also known as the indirect *antiglobulin test* (IAT), involves testing the patients' serum against reagent red blood cells selected to possess relevant blood group antigens for detection of clinically significant, unexpected antibodies. The term “*unexpected antibodies*” refers to those antibodies other than anti-A or anti-B. It is expected that a group A person will have anti-B in their serum. The lack of expected ABO antibodies will result in the need for additional testing. *Clinically significant antibodies* are of the IgG class and react in-vitro, preferentially at 37 C or at the anti-human globulin phase of testing. These antibodies were determined to be clinically significant if they have been responsible for hemolytic transfusion reactions or hemolytic disease of the newborn. Simply stated, they are responsible for red blood cell destruction. In-vitro hemolysis and/or agglutination of the screen cells by the patient serum/plasma at any of the various phases of testing constitutes a positive test result and indicate the presence of unexpected antibodies which could possibly *cause in-vivo hemolysis which may manifest as decreased survival of transfused red blood cells or hemolytic disease of the newborn*. Antibodies that are reactive at 37C and/or in the anti-human globulin test are more likely to be significant than those reactive at room temperature or below. A positive reaction is obtained in the antibody screen will result in the need for addition testing to determine the specificity of the antibody so that appropriate antigen negative donor units can be selected.

The absence of hemolysis and/or agglutination constitutes a negative test indicating the serum/plasma being tested does not contain *detectable* antibodies directed to any of the wide variety of antigens present on the reagent screen cells.

Although two cell screens are available a three cell screen is recommended, especially for those facilities who routinely utilize a Type and Screen (T&S) instead of a crossmatch or who perform an abbreviated crossmatch procedure (the abbreviated crossmatch will be discussed in Lab 6). A three cell screen provides cells which are homozygous for the antigens to which individuals most frequently make antibodies. This helps ensure the detection of weakly reactive antibodies. Some antibodies are so weak that they may show a *dosage effect*. In dosage effect the antibodies may react most strongly or **ONLY** with cells which are homozygous (have a double dose of the antigen, ie, $Fy^{a+}Fy^{b-}$) and give negative or weakly reactive results with heterozygous cells (have single dose of antigen, ie, $Fy^{a+}Fy^{b+}$). The heterozygous cells possess half as much antigen as the homozygous cells which may result in weak or negative reactions.

The screening cells are supplied as three vials, each containing a suspension of human **group O** red blood cells derived from a **single donor**. These red blood cells have been phenotyped for the antigens to which

patients most frequently make antibodies to. The results of these typings are provided on a piece of paper known as an *Antigram*. A “+” in the column under the antigen indicates the cell has been tested and found to be positive for the antigen, a “0” indicates the cell has been tested and found to be negative for the antigen. It is *critically important* that the lot number on the Antigram match the lot number on the screen cells. Each lot number of screen cells will have different donors which will change the antigenic make up.

In some laboratories the patient's red cells are tested with their serum as an *autologous (auto) control*, by the same method used for the antibody detection test. This procedure is done to detect antibodies which are coating red cells in the patient's circulation. AABB Standards does not require that an autologous control be done. It has been found that even when it is positive, it is of limited value in pretransfusion testing. When the auto control is positive additional testing must be performed (DAT, possibly an eluate, etc). The information obtained from these tests rarely changes the selection of blood for transfusion when the antibody screen is negative and usually causes an unnecessary delay in getting blood to the patient. Auto controls **are** routinely performed when an antibody workup is done.

Principle

This procedure consists of two (2) parts:

1. Performing a complete ABO and D typing on the patient's blood sample.
2. Screening the patient's serum for atypical antibodies using 2-3 reagent screen cells.

The principle of the ABO and D typing procedure was covered in Exercise 3, please review it.

Starting with this procedure and for all future procedures requiring an ABO/D type, you will set up a second set of forward typing tubes for a retype. AABB requires that all patients without a history have a second ABO/D typing done as part of the testing process. Most institutions perform batch testing which results in the potential for the wrong serum/cell tubes to be placed in front of the wrong clot tubes. This could result in a serious ABO typing error. To prevent this from happening two sets of forward typing tubes are set up, reagents added, then one set of tubes will receive drops of blood from the washed cell suspension while the second set of tubes will receive drops of blood from the primary clot tube. If a discrepancy occurs between the two sets of tubes then testing must be repeated.

The principle of the antibody screen is as follows: well mixed antibody screen cells of known phenotypes are added to patient serum and are evaluated/read at three different phases to determine if there is an unexpected antibody in the patient's serum which is reacting with a blood group antigen present on the reagent screen cells constituting a positive reaction.

You must memorize each phase of the testing procedure and the antibody class most commonly detected by each.

Saline Immediate Spin (IS) Phase

This phase provides conditions that best allow the detection of IgM class antibodies. Due to their structure, antibodies of the IgM class readily agglutinate red cells suspended in saline. Patient serum is added to the 3 screen cells, the tubes are immediately spun down and observed for hemolysis and/or agglutination by **gently** resuspending the cell button. If a positive reaction is obtained at this phase, **proceed to the incubation and antiglobulin phase** to see if the reaction goes away or gets stronger.

Reactivity at IS only usually indicates the presence of a “nuisance” cold reactive antibody. Additional testing would be necessary to confirm this.

Albumin, 37°C Incubation Phase

Bovine serum albumin or some other additive is used to enhance agglutination of red blood cells by antibodies of the IgG class. One theory states that the albumin reduces the zeta potential (electrical charges) between the red blood cells, allowing them to come closer together. This may allow the small IgG molecules to bridge the gap between the red cells allowing lattice formation (agglutination) to occur. Albumin affects the second stage of agglutination. There are several other enhancement medias used routinely in the blood bank, LISS, PEG and others. You will gain experience with some of these during your clinical rotation.

The IgG antibodies react optimally at 37°C. If an antibody is reactive at this phase in vitro (in the test tube) then it may also react in vivo (in the body).

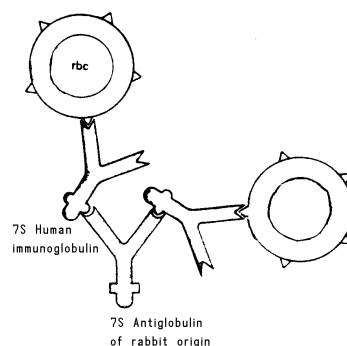
After the incubation phase, all tubes are again spun down and observed for agglutination and/or hemolysis by gently dislodging the cell button and record the graded reactions. If a positive or negative reaction is obtained at this phase proceed to the AHG phase.

Anti-human Globulin Phase (AHG or Coombs)

Many antibodies of the IgG class are small and do not always cause visible agglutination. During the incubation phase, the antibodies may attach to antigens on individual red cells (*sensitize*) but, due to their small size, cannot cause agglutination.

To determine if sensitization has occurred, all tubes are washed three times with saline to remove the serum and albumin. Antihuman globulin (AHG or Coomb's) serum is added to all tubes. AHG is an anti-antibody which will attach to antibodies coating the cells. The tubes are spun down and again evaluated for agglutination both macroscopically and microscopically. This is the **most important phase** of the procedure because most clinically significant antibodies are of the IgG class and **only** react at Coombs.

It is critical that you memorize the principle of this test and be able to create a drawing to illustrate the principle. Some clinical instructors will require you to do this as part of your blood bank rotation.



Model of antiglobulin agglutination of cells coated with 7S (incomplete) antibodies.

Coombs Control Cells (Check Cells)

There are many potential sources of error in the Coomb's procedure. Washing must be thorough to remove all unbound antibody. *As little as one drop of a 1:4000 dilution of human serum can neutralize one drop of Coombs serum.* Human serum contains IgG antibodies to any disease or vaccine which we have become immune to. Failure to wash sufficiently or resuspend the cell button in between washes may result in unbound IgG in the test tube. The free IgG will preferentially bind to the anti-IgG (AHG) which result in neutralization of the reagent. To ensure that this has not occurred, and that the Coomb's serum is active, Coombs control cells (check cells) are added to each negative reacting tube. Coombs control cells are commercially prepared, sensitized reagent red blood cells (cells coated with IgG antibody). If the Coombs serum is active, and has not been neutralized, a positive reaction will be obtained. ***If a negative reaction is obtained after the check cells are added, the test procedure is invalid and must be repeated.*** The most **common** cause of check cells not “checking” is insufficient washing.

Refer to your Lecture Guide and textbook for the many causes of false positive and false negative reactions at the AHG phase (Coomb's procedure). You **MUST** know the possible causes of false negative/positive reactions. This will enable you to problem solve and trouble shoot unexpected problems encountered in the testing procedure.

Limitations of the Procedure

In all serological tests, such factors as contaminated materials, improper incubation or temperature, improper centrifugation or improper examination for agglutination may give rise to false results. Other limitations of antibody detection and identification tests include:

1. The use of plasma or aged serum may result in failure to detect complement dependent antibodies.
2. The antibody in the serum being tested may be directed at an antigen not represented on the reagent red blood cells (low incidence antigen).
3. The patient may have antibodies to components of the preservative solution in the reagent screen cells.
4. The patient may have antibodies to a component in the bovine albumin.

Reagents

1. See page 1 of procedure manual.
2. New Reagents - Trio Screen Cells

Procedure

A. Specimen Preparation and Rack Set-up

1. Label two tubes with the patient's complete name and hospital number. If the serum is already in a tube, properly label it.
2. Place approximately five (5) drops of cells in the second tube. Place a typenex sticker (strip of yellow stickers with 3 letters and 4 numbers) on this tube.
3. Fill the tube containing the red cells with saline, spin for one minute, using the serum tube for a balance tube. Decant the saline completely and add enough saline back to make a 4-6% cell suspension.
4. While the serum and cells are spinning, label tubes and arrange in test tube rack as shown.
STARTING WITH THIS PROCEDURE you must label a set of tubes for the patient repeat forward type. Place this second set of tubes directly behind the first set.

<i>Use RBCs from clot</i>		R-A	R-B	R-A,B	R-D							
Clot	Pt RBCs	anti-A	anti-B	anti-A,B	anti-D		Pt Serum	A1 cells	B cells	S1	S2	S2

REMEMBER: Patient initials at the top of *each* tube. Patient's **full name and hospital number** on serum and cells.

B. Type and Screen

1. Add one (1) drop of each appropriate reagent anti-sera to its properly labelled tube.
2. Add three (3) drops of patient serum to S1, S2, S3, A cell and B cell tubes.
3. **Visually inspect all tubes at this time to make sure that all serums have been added.**
4. Add one (1) drop of the *patient washed cell* suspension to the forward typing tubes.
5. Add 1 drop of cells *from the original clot* tube to the "retype" tubes.
6. Add one (1) drop of *reagent A, B, S1, S2 and S3* cells to the appropriately labelled tubes.
7. Spin S1, S2 and S3 tubes in the serofuge for 15 seconds.
8. Read each of these tubes for agglutination and/or hemolysis. **Record results as each tube is read.**
9. Add two (2) drops of 22% bovine albumin to S1, S2 and S3 tubes and place in 37°C water bath for 20 minutes.
10. Spin down the forward and reverse ABO and D and the repeat ABO/D typing tubes for 15 seconds.
11. Read for agglutination. **Record these results now.** If the patient appears to be D negative set up the D ctrl tube and **immediately** place the anti-D and D ctrl tubes in the 37°C water bath for 15 minutes (if this is done quickly the incubation should be completed at the same time as the antibody screen).
NOTE: If patient appears to be AB positive, an D control must be run.
12. After the 20 minutes incubation, spin the S1, S2 and S3 for 20 seconds.

13. Read each tube for agglutination and/or hemolysis. **Record results as each tube is read.**
14. Wash the S1, S2 and S3 (and anti-D and D ctrl tube if your patient appeared to be D negative) three (3) times with saline.

AHG Phase Washing Procedure Notes

- a. *Washing process must be carried out in the shortest time possible.*
 - b. *Completely resuspend cell buttons after each wash, visually inspect bottom of tubes after addition of saline, if a rbc button is visible resuspension was not achieved and an additional wash should be performed.*
 - c. *Spin time is one minute for each wash.*
 - d. *Completely decant saline between each wash, turn tubes over and allow saline to drain, do not shake the tubes as they are draining, this will result in the loss of your rbcs.*
 - e. *After the third wash, decant completely and **blot the rims of the tubes dry with biowipe to produce a dry cell button.***
15. Add two (2) drops of anti-human globulin (AHG) reagent to each tube, resuspend the cells completely and spin for 15 seconds. **NOTE:** *Make sure you are adding AHG, which is green, and NOT Coombs control cells, which is a cell suspension!*
 16. Read for agglutination macro- **and** microscopically. Record results after reading micro.
 17. To all tubes showing a negative reaction add one (1) drop of Coombs control (check) cells. Mix well and spin for 15 seconds.
 18. Read for agglutination macroscopically. A 1-2+ reaction must be obtained or all results are invalid. Record results by placing a check in the check cell column if agglutination was obtained.

NOTE: Some facilities may still run an auto control (patient serum and patient cells) as part of the antibody screen. This is no longer recommended in routine testing, but must be part of an antibody workup.

Interpretation of Results

If all three (3) screen cells give negative results throughout all three phases of testing, the interpretation of the test is “negative”.

If one (1) or more of the screen cells is positive (agglutination and/or hemolysis of the test rbc's has occurred), the interpretation of the antibody screen is “positive.” This is indicative of an antibody present in the patient's serum reacting with an antigen present on the screen cells. Additional testing is necessary to determine the specificity of the antibody and to find appropriate units to crossmatch.

Recording Results

Clerical errors will be counted off heavily as well as misinterpretation of results. Remember, the most common cause of transfusion related deaths is due to the patient receiving ABO incompatible blood and this is almost always due to clerical errors. Fill the form in carefully as points will be deducted for all clerical errors. ***Record each tube as you read it.***

Terms Utilized

The following terms may be used interchangeably. *Memorize* these terms:

Indirect Antiglobulin Test

- IAT
- antibody screen
- screen
- Indirect Coombs

IgG Antibodies

- immune antibodies
- incomplete antibodies
- clinically significant antibodies
- coating antibodies
- sensitizing antibodies
- unexpected antibodies
- atypical antibodies

IgM Antibodies

- naturally occurring antibodies
- complete antibodies

Anti-human Globulin Serum

- AHG serum
- Coombs serum

Student Name: _____

Exercise _____

Date: _____

Name	Anti A	Anti B	Anti A,B	Anti D	D Ctrl *	D ^u	✓	D ^u Cont **	✓	A ₁ Cells	B Cells	Interp.	DAT			Elution Results	FBS		
													Poly	IgG	C ³		Pos	Neg	Pt
ID #																			
Retype																			

BB#	Rack#	Collected	Completed	Tech	Screen 1			✓	Screen 2			✓	Screen 3			✓	Interp.	✓	Antibody ID
					IS	37°	AHG		IS	37°	AHG		IS	37°	AHG				

Remarks

	Blood Bank #	Donor #	Anti A	Anti B	Anti A, B	Anti D	D Cont	D ^u *	✓	D ^u Ctrl **	✓	Donor Type & Rh	IS	37°	AHG	✓	Interp.	Tech	Date	Time	
#1																					
#2																					
#3																					
#4																					
#5																					

*performed when patient/donor appears to be AB positive or D negative
 **If a weak (positive D^u) is obtained, a D^u control or DAT must be performed.

EXERCISE 5 Type & Screen

Laboratory Procedure Manual

Student Name: _____

Exercise _____

Date: _____

Name	Anti A	Anti B	Anti A,B	Anti D	D Ctrl *	D ^u	✓	D ^u Cont **	✓	A ₁ Cells	B Cells	Interp.	DAT			Elution Results	FBS		
													Poly	IgG	C ³		Pos	Neg	Pt
ID #																			
Retype																			

BB#	Rack#	Collected	Completed	Tech	Screen 1			✓	Screen 2			✓	Screen 3			✓	Interp.	✓	Antibody ID
					IS	37°	AHG		IS	37°	AHG		IS	37°	AHG				

Remarks

	Blood Bank #	Donor #	Anti A	Anti B	Anti A, B	Anti D	D Cont	D ^u *	✓	D ^u Ctrl **	✓	Donor Type & Rh	IS	37°	AHG	✓	Interp.	Tech	Date	Time
#1																				
#2																				
#3																				
#4																				
#5																				

*performed when patient/donor appears to be AB positive or D negative
 If a weak (positive D^u) is obtained, a D^u control **or DAT must be performed.

7. State the principle of the antibody screen (Indirect Antiglobulin Test/IAT). (1 point)
8. Describe “dosage effect” as it relates to positive reaction in the IAT. (1 point)
9. Based on your knowledge of the ABO blood group system state why the donors used for screen cells are group 0. (1 point)
10. Describe the “auto control” and state the reason that it is of limited value in routine pretransfusion testing. (1 point)
11. List the three phases of the IAT **AND** state the antibody class most commonly detected. (3 points)

Phase	Antibody Class Detected
a.	
b.	
c.	

12. Define “zeta potential”. How does bovine albumin affect the zeta potential? (1 point)
13. State the principle of the *anti-human globulin* test and draw a picture to illustrate this principle. (2 points)
14. Describe the reagent “check cells” and explain the importance of this procedure. What is the most common reason for check cells not “checking”? (1.5 points)
15. There are many reasons or causes of false positive and false negative reactions occurring during the Coomb’s procedure. Based on information provided in your textbook, lecture guide and in this lab, list three (3) reasons for false positive reactions **AND** three (3) reasons for false negative reactions. (3 pts)

False Positive

a.

b.

c.

False Negative

a.

b.

c.

16. A patient has a positive antibody screen. State why this patient would not be a T&S candidate. (1 point).

17. For each of the following state at least 1 alternative terms which may be used: (2 points)

IAT

IgG antibodies

IgM antibodies

AHG serum