

## Exercise 7

## Panel Studies

Textbook: Quinley, Chapters 8, 9 and 10

Skills: 30 points

Objectives:

1. Perform a 2 unit crossmatch with 100% accuracy.
2. Determine with 100% accuracy the allo-antibody present in the patient specimen.
3. State the percentage of the population which may have unexpected antibodies present.
4. State the test which must be performed if a patient has a positive antibody screen.
5. Define “clinically significant” antibodies as the term applies to immunohematology.
6. State the importance of identifying unexpected antibodies in a patient sample.
7. State the make up of a panel, including blood group and antigen typing.
8. State the principle of the antibody identification (panel) test.
9. List two observations of a positive serological result.
10. List the criteria for a panel to be functional during antibody identification.
11. State the criteria for conclusive identification of an unexpected antibody.
12. List 3 serologic characteristics/observations which may be helpful in evaluating a panel.
13. List 6 general characteristics of blood group antibodies that apply to specific blood group antibodies.
14. List 5 methods, and the purpose for using, which may be utilized to enhance antigen-antibody reactions in-vitro.
15. Given a completed panel
  - a. identify the primary antibody specificity present.
  - b. State the clinical significance of the identified antibody.
  - c. State whether units must be phenotyped for the antigen.
  - d. State the expected outcome of the patient’s phenotype and compatible donor units.
  - e. determine which antibodies may be masked or covered up by the primary antibody
  - f. Determine which of the possible antibodies in b need additional work.

### Discussion

AABB Standards requires a screening procedure to detect unexpected alloantibodies in the serum of patients who are candidates for transfusion. Alloantibodies are demonstrable in the serum of *approximately 0.3 to 2% of the population*, depending upon the group of individuals examined and the techniques employed for detection. For example, if a technician is employed in the blood bank of a large cancer hospital where patients are frequently transfused, the incidence of detecting unexpected antibodies is higher than in a small general hospital where antibody problems are seen with much less frequency.

Once it has been determined that an *antibody screen is positive*, a *panel study must be done* to determine the specificity of the antibody present. One very important reason for *identifying the specificity of an unexpected antibody is to ensure that blood selected for a needed transfusion to that patient is negative for the corresponding antigen*. **Clinically significant** antibodies have the potential of causing acute hemolysis if antigen positive donor cells are transfused. The crossmatch procedure performed using the recipient patient's serum (with the antibody) and the antigen positive donor cells may not demonstrate incompatibility if the antibody in the recipient's serum sample is weak. Knowing the identity of the antibody makes it possible to

select potent reagent antiserum of the same specificity, to test donor bloods for the presence of the antigen(s) in question.

### Principle

The principle of the panel is similar to that of the antibody screen. Patient serum is tested against a panel of eight or more group O reagent cells of known antigen composition. Most labs use commercial panels which provide a list showing in moderate detail the antigenic composition of each cell. *Hemolysis and/or agglutination* of a cell at any stage of the test is a positive result indicating that the antibody(ies) is/are reacting with an antigen(s) on that cell. The absence of hemolysis/agglutination of a particular cell at any stage means that the cell did not possess the antigen(s) for which the antibody(ies) was/were directed.

It is important to keep in mind that this procedure makes use of the presence of antigens (both homozygous and heterozygous) as well as their absence on individual reagent cells to identify the specificity of an unexpected antibody in the patient's serum.

***A panel, to be functional, must make it possible to identify with confidence the most commonly encountered antibodies (e.g., anti-D, anti-K, anti-E, and anti-Fy<sup>a</sup>) and exclude the possible presence of most other antibodies.*** A distinct pattern should be apparent for most examples of single antibodies. For example, the only two Kell positive cells should not also be the only cells positive for E.

For conclusive antibody identification, there must be enough cells positive and negative for each antigen that the results of serum testing can give a definitive pattern not caused by chance alone. *An acceptable probability of identification should show at least three (3) cells positive which react with the patient's serum and three (3) cells negative for the antigen which do not react with the patient's serum at which the antibody is directed.*

Antibodies of certain specificities tend to have ***consistent serologic characteristics***. In interpreting panel results, it is desirable to look for those characteristics, in addition to observing which cells are positive or negative. The following *observations* may be helpful.

1. The effect of temperature, suspending medium, or enzymes on the reactions with a specific cell.
2. Variation in the strength of reaction among positive cells (dosage affect).
3. Presence of hemolysis.

A review of information on the *general characteristics of blood group antibodies* will also be helpful.

1. *Reactivity at RT IS phase.* The antibodies most often identified by a cold panel are Lewis, Lu<sup>a</sup> I, M, N and P1.
2. *Reactivity at 37 C.* Antibodies most often identified after incubation at 37 C are Rh, anti-K, -S and -s.
3. *Reactivity at AHG.* After addition of antiglobulin serum, almost all warm antibodies can be detected: Rh antibodies, anti-K, -Jk, -Fy, -S, etc.
4. *Hemolysis.* Antibodies most often hemolyzing saline suspended cells are anti-Lea and Kidd antibodies.
5. *Affect of enzymes.* Anti-Fy, -M, -N and -S may not be detected when enzymes are used. These *antigens* are denatured or destroyed by enzymes. This technique is very useful when multiple antibodies are present and one appears to have one of these specificities. Rh antibody reactions are enhanced by the enzyme procedures in common use.

6. *Alteration of pH.* The reactivity of some weak examples of anti-M are greatly enhanced when the pH of the serum is lowered.

In doing a panel study, it is often advantageous to ***use methods to enhance the antigen-antibody reaction.*** This is especially important when an antibody gives a  $\pm$  to 1+ reaction. The following technique variations are methods that may be used to enhance the antigen-antibody reaction. The selection of which, if any, to use will depend on previously gathered information, such as how the antibody reacted during the initial antibody screen.

1. Increasing serum to cell ratio to increase the amount of antibody present and increase the strength of the reaction (use 4 or 5 drops of serum instead of 3).
2. Increase the incubation time to allow more antibody to react with more antigen sites to increase the strength of the reaction (increase incubation time from 20 minutes to 30 minutes).
3. Change incubation temperature. While some antibodies prefer room temperature, others such as anti-P<sub>1</sub> prefer 4°C. It is very important to run an auto control when running tests in the cold.
4. Enhancement media. Low ionic strength salt solutions (LISS) have been shown to enhance agglutination and antibody uptake, but they require experience to work with.
5. Use fresh serum specimen. Some rare examples of Kidd antibodies will only react in the presence of complement, as serum ages the complement levels decrease rapidly.

<b>Reagents</b>	1.	See page 1
	2.	Reagent panel

**Procedure** 1 Perform a 2 unit crossmatch on patient specimen. If antibody screen is positive, go to #2. If antibody screen is negative, see instructor.

**NOTE:** In “*real*” life a panel would not be performed unless a positive antibody screen was obtained. Due to the time limitation in the student laboratory setting you will set up the panel as soon as you are finished setting up the crossmatch.

2. Label 12 tubes with the patient's first and last initials and the numbers 1-11, tube #12 is labeled with patient initials and “AC” for auto control.
3. Add one (1) drop of each reagent panel cell to the appropriately labelled tube. One (1) drop of patient cells will go in tube #12.
4. Add four (4) drops of patient serum to each tube.
5. Spin for 15 seconds. *Put the tubes in the serofuge in the order they will be read.*
6. Observe for agglutination and/or hemolysis. **Record reactions as each tube is read.**

**IMPORTANT:** You will write your reactions on the panel antigram sheet. Make sure that the lot number on the vials of cells used matches the lot number on the panel sheet. Each lot number of panel cells has a different antigenic make-up, so it is critical that you pick up the antigram which matches the lot number of panel cells used.

7. Add two (2) drops of bovine albumin, mix well and incubate for 30 minutes at 37°C.
8. After incubation, spin all tubes for 20 seconds.
9. Observe each tube for agglutination and/or hemolysis. **Record reactions as each tube is read.**
10. \*Wash cells three (3) times, decanting completely and resuspending cell button between washes.
11. \*After the third wash blot tubes dry to obtain a dry cell button.
12. Add two (2) drops of Coombs sera and spin all tubes for 15 seconds.
13. Read for agglutination. All tubes showing a negative result must be read microscopically. **Record reaction as tubes are read.**
14. Add one (1) drop of check cells to all tubes showing negative reactions and spin 15 seconds.
15. A 1 - 2+ agglutination must be obtained or the entire test must be repeated.

**Interpretation** Interpreting the results of a panel study requires practice. Upon completion of the testing phases, a pattern of positive and negative reactions will be obtained.

When interpreting a panel, a systematic approach must be used to evaluate the reaction given by each reagent cell. For example, if the antibody in the patient's serum does not react with reagent cell #1, any antigen listed on the antigram sheet (included with the panel) that is positive or present on that cell can be crossed out and eliminated from consideration. This process is continued for each reagent cell giving a negative reaction. After all negative reacting cells have been evaluated, it is often possible to easily identify the specificity of the antibody by comparing the reaction pattern of the antibody with the positive and negative patterns of antigens that have not been crossed out.

It is of critical importance to evaluate each negative reacting cell in this manner even when the reactions have a very clear pattern indicating one antibody. The primary antibody may mask or cover up additional antibody specificities. For example, if a patient has anti-e there may be a number of potential antibodies that may be masked as there are only 2 e negative cells on the panel. Perform the following steps to ensure the identification of the antibody.

1. Evaluate each negative cell.
2. Identify the primary antibody (the one which fits the pattern perfectly) and all possible underlying antibodies.
3. Evaluate the clinical significance of the possible underlying antibodies.
4. If a possible underlying antibody **is** clinically significant perform additional testing to prove its presence or absence.

An antibody is considered “clinically significant” if it has the potential of causing hemolysis in-vivo. Antibodies which react at room temperature **only** are generally considered to be clinically insignificant and include the following: anti-Le, -P<sup>1</sup>, -I, -M, -N, and Lu<sup>a</sup>. These are most commonly antibodies of the IgM class.

Clinically significant antibodies react in-vitro at 37°C and/or the AHG phase. These are of the IgG class and include the following: all Rh, Kidd (Jk), Duffy (Fy), Kell (K), S,  $\bar{s}$  and Lutheran<sup>b</sup> (Lu<sup>b</sup>) antibodies.

It is important to remember that some antibodies are very weak and may react with homozygous cells, but not with heterozygous cells. If after crossing out, the antibody still cannot be identified, start looking for a dosage pattern (all homozygous cells are reactive, while heterozygous cells are not).

Once an antibody has been identified, phenotype the patient for the antigen. This is to conclusively prove that the patient is negative for the antigen, since patients rarely form antibodies against antigens on their own red cells. If a patient has been *recently transfused*, the cells **cannot** be antigen typed unless a method is used to separate patient cells from donor cells, since the transfused donor cells may still be circulating and may be antigen positive. If the patient is to be transfused, the donor units **must** be typed for the antigen **even if the units appear crossmatch compatible**.

Name

### Interpretation of Results - Pulling it all together

1. What is the primary antibody specificity?
2. What other antibodies may be masked or covered up by the primary antibody?
3. If any antibodies were listed in #2 which would need additional testing to confirm or rule out their presence?
4. What additional testing must now be performed on the patient and donors?
5. What is the expected results? (Look at crossmatch results to determine part of the answer to this question).
6. Look at the antigram for your screen cells. Does this antibody specificity detected in the panel match the antibody specificity of the screen cells?
7. Which screen cell would you select as a positive control for antigen testing? Negative control?

Name \_\_\_\_\_

**Exercise 7****Panel Studies  
Study Questions**

1. What test procedure is done to follow-up a positive antibody screen (IAT)? (0.5)
2. Why is it so important to identify the specificity of an antibody in a transfusion candidate? (1 point)
3. What problem may occur in a patient with a clinically significant antibody who is transfused with antigen positive blood? (0.5)
4. List least four (4) methods that can be used to *enhance the strength of antigen-antibody reactions* of the test system. (2 points)
5. In a panel study, what is an acceptable probability of identification? (1 point)

6. What percentage of the population will have demonstrable alloantibodies in their serum? (0.5)
  
7. In order to be functional, what must a panel be able to prove? (1 point)
  
8. Which antibody class reacts at room temperature? Which antibody class reacts in the Coombs phase? (1 point)
  
9. An antibody *has been identified* in a patient needing a blood transfusion, what additional testing must be performed on the patient **and** donors. What is the expected outcome of this test? (1.5 point)
  
10. Antibodies of certain specificities tend to have *consistent serologic characteristics*. Briefly state 3 observations which may be helpful in antibody identification. (1.5 points)
  
11. List the antibody specificities which react preferentially at RT or below. (2 points)

12. List the antibody specificities which react at 37C and/or AHG. (2 points)
  
13. List the antibodies which may cause in-vitro hemolysis. (1 point)
  
14. List the antigens which are denatured or destroyed by enzymes. State how this is useful. (2 points)
  
15. State the antibody which has enhanced activity when the pH is lowered. (0.5)
  
16. List the antibodies which have enhanced reactivity when tested with enzyme treated cells. (0.5)
  
17. State the antibody which may be missed if fresh complement is not present in the serum. (0.5)
  
18. Briefly describe the process used to interpret a panel study. (2.5)

19-23. Refer to the attached panels number 19-23. In each, one antibody can be identified. Using the interpretation method demonstrated in lab, record your interpretation in the space provided. Each “primary antibody specificity” is worth 1 point. “Unable to rule out” varies with number listed.

	<b>Primary Antibody Specificity</b>	<b>Unable to Rule Out</b>
19.		
20.		
21.		
22.		
23.		

**EXERCISE 7**

**Laboratory Procedure Manual**

#19

	BLOOD GROUP SYSTEM				Rh						MNSs					P	Lewis		Lutheran		Kell				Duffy		Kidd		Sex Linked		SPECIAL TYPE	I3	30" Alb 39"	IgG			
	Rh	Code	D	C	E	c	e	f	V <sup>+</sup>	VS <sup>+</sup>	C <sup>w</sup>	M	N	S	s	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	Lu <sup>a</sup>	Lu <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	X <sub>a</sub> <sup>g</sup>	Sex							
1	r'r	-5234	0	+	0	+	+	+	0	/	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	+	+	+	F		0	0	3+		
2	R1R1w	-4114	+	+	0	0	+	0	0	/	+	0	+	0	+	0	0	+	0	+	0	+	0	0	0	+	+	+	+	/		0	0	3+			
3	R1R1	-158	+	+	0	0	+	0	0	0	0	+	+	0	+	0	0	0	+	+	W	+	0	0	+	+	0	+	M		0	0	3+				
4	R2R2	-181	+	0	+	+	0	0	0	0	0	+	+	+	0	0	+	0	+	0	+	0	0	0	+	+	+	0	M		0	0	0	✓			
5	r''r	-4053	0	0	+	+	+	+	0	0	0	+	+	+	0	0	+	0	+	0	+	0	0	+	+	+	+	+	M		0	0	0	✓			
6	rrV	-22	0	0	0	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	0	F		0	0	0	✓			
7	rrK	-5563	0	0	0	+	+	+	0	/	0	+	+	+	0	+	+	0	0	+	+	+	0	0	+	+	+	0	/		0	0	0	✓			
8	rrJsa	-10	0	0	0	+	+	+	0	0	0	+	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	+	F		0	0	0	✓			
9	rr	-4369	0	0	0	+	+	+	0	/	0	+	+	+	0	+	+	0	0	+	0	+	0	0	+	+	0	+	/		0	0	0	✓			
10	rr	-37	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	0	0	+	+	0	+	M	Rg-	0	0	0	✓			
11	R2R2	-191	+	0	+	+	0	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	0	0	+	+	+	F	Sc:1,2	0	0	0	✓			
																																			✓		
	A	B	A,B	A <sup>1</sup>	H	D	C	E	c	e	f	V <sup>+</sup>	VS <sup>+</sup>	C <sup>w</sup>	M	N	S	s	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	Lu <sup>a</sup>	Lu <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	X <sub>a</sub> <sup>g</sup>	Sex				
																			Reverse Group Cells		A <sub>1</sub>																
																					A <sub>2</sub>																
																					B																





**EXERCISE 7**

**Laboratory Procedure Manual**

#22

BLOOD GROUP SYSTEM		Rh								MNSs					P	Lewis		Lutheran		Kell				Duffy		Kidd		Sex Linked		SPECIAL TYPE	I3	Alb 37° 30"	IgG				
Rh	Code	D	C	E	c	e	f	V <sup>+</sup>	VS <sup>+</sup>	C <sup>w</sup>	M	N	S	s	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	Lu <sup>a</sup>	Lu <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	X <sub>g</sub>	Sex								
1	r'r	-5234	0	+	0	+	+	+	0	/	0	+	+	0	+	0	0	+	0	+	0	+	0	+	0	0	0	+	+	0	+	F		0	0	0	✓
2	R1R1w	-4114	+	+	0	0	+	0	0	/	+	0	+	0	+	0	0	+	0	+	0	+	0	0	0	+	+	+	+	/		0	0	0	✓		
3	R1R1	-158	+	+	0	0	+	0	0	0	+	+	0	+	+	0	0	0	+	+	W	+	0	0	+	+	0	+	M		0	0	3+				
4	R2R2	-181	+	0	+	+	0	0	0	0	0	+	+	+	0	0	+	0	+	0	+	0	0	0	+	+	0	+	M		0	0	0	✓			
5	r''r	-4053	0	0	+	+	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	+	+	+	M		0	0	0	✓			
6	rrV	-22	0	0	0	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	0	F		0	0	0	✓			
7	rrK	-5563	0	0	0	+	+	+	0	/	0	+	+	+	0	+	+	0	0	+	+	+	0	0	+	+	+	0	+	/		0	0	3+			
8	rrJsa	-10	0	0	0	+	+	+	0	0	+	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	F		0	0	0	✓			
9	rr	-4369	0	0	0	+	+	+	0	/	0	+	+	+	0	+	+	0	0	+	0	+	0	0	+	+	0	+	/		0	0	0	✓			
10	rr	-37	0	0	0	+	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	0	0	0	+	+	0	+	M	Rg-	0	0	0	✓			
11	R2R2	-191	+	0	+	+	0	0	0	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	+	+	+	+	F	Sc:1,2	0	0	0	✓			
																																			✓		
	A	B	A,B	A <sup>1</sup>	H	D	C	E	c	e	f	V <sup>+</sup>	VS <sup>+</sup>	C <sup>w</sup>	M	N	S	s	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	Lu <sup>a</sup>	Lu <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	X <sub>g</sub>	Sex				
																												Reverse Group Cells	A <sub>1</sub>								
																													A <sub>2</sub>								
																													B								

**EXERCISE 7**

**Laboratory Procedure Manual**

#23

	BLOOD GROUP SYSTEM		Rh							MNSs					P	Lewis		Lutheran		Kell				Duffy		Kidd		Sex Linked		SPECIAL TYPE	I3	Alb 37° 30"	IgG				
	Rh	Code	D	C	E	c	e	f	V <sup>+</sup>	VS <sup>+</sup>	C <sup>w</sup>	M	N	S	s	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	Lu <sup>a</sup>	Lu <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	X <sub>a</sub> <sup>g</sup>						Sex		
1	r'r	-5234	0	+	0	+	+	+	0	/	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	+	+	+	F		2+	0	0	✓	
2	R1R1w	-4114	+	+	0	0	+	0	0	/	+	0	+	0	+	0	0	+	0	+	0	+	0	0	0	+	+	+	+	/		0	0	0	✓		
3	R1R1	-158	+	+	0	0	+	0	0	0	0	+	+	0	+	0	0	0	+	+	W	+	0	0	+	+	0	+	M		2+	0	0	✓			
4	R2R2	-181	+	0	+	+	0	0	0	0	0	+	+	+	0	0	+	0	+	0	+	0	0	0	+	+	+	+	M		0	0	0	✓			
5	r''r	-4053	0	0	+	+	+	+	0	0	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	+	+	M		4+	2+	0	✓			
6	rrV	-22	0	0	0	+	+	+	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	0	0	0	F		0	0	0	✓			
7	rrK	-5563	0	0	0	+	+	+	0	/	0	+	+	+	0	+	+	0	0	+	+	+	0	0	+	+	+	0	+	/		2+	0	0	✓		
8	rrJsa	-10	0	0	0	+	+	+	0	0	0	+	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	0	F		2+	0	0	✓			
9	rr	-4369	0	0	0	+	+	+	0	/	0	+	+	+	0	+	+	0	0	+	0	+	0	0	+	+	0	+	/		2+	0	0	✓			
10	rr	-37	0	0	0	+	+	+	0	0	0	+	0	+	+	0	+	0	0	+	0	+	0	0	0	+	+	0	M	Rg-	4+	2+	0	✓			
11	R2R2	-191	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	+	+	+	F	Sc:1,2	0	0	0	✓			
																																			✓		
	A	B	A,B	A <sup>1</sup>	H	D	C	E	c	e	f	V <sup>+</sup>	VS <sup>+</sup>	C <sup>w</sup>	M	N	S	s	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	Lu <sup>a</sup>	Lu <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	X <sub>a</sub> <sup>g</sup>	Sex				
																			Reverse Group Cells		A <sub>1</sub>																
																					A <sub>2</sub>																
																					B																