Exercise 7

Panel Studies

Skills: 30 points

Objectives:
1. Perform a 2 unit crossmatch with 100% accuracy.
2. Determine with 100% accuracy the alloantibody 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 makeup 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 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 the purpose of 5 methods, which may be utilized to enhance antigen-antibody reactions in-vitro.
15. Given a completed panel-
   - Identify the primary antibody specificity present.
   - State the clinical significance of the identified antibody.
   - State whether units must be phenotyped for the antigen.
   - State the expected outcome of the patient’s phenotype and compatible donor units.
   - Determine which antibodies may be masked or covered up by the primary antibody.
   - 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*) 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).

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^a 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 Kidd antibodies.
5. Effect 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 +/- 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 2).
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-P₁ prefer 4°C. It is very important to run an auto control when running tests in the cold.
4. 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.
Reagents
1. Blood Bank Reagent Rack
2. Reagent Panel

Procedure
1. Perform a 2 unit crossmatch on the patient specimen provided by the instructor. If antibody screen is positive, go to step number two. If antibody screen is negative, see instructor.

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 patient autocontrol.

3. Add two (2) drops of patient serum to each tube.

4. Add one (1) drop of each reagent panel red blood cell to the appropriately labelled tubes. Add one (1) drop of patient cells to the AC.

5. Spin for 15-20 seconds. Put the tubes in the serofuge in the order they will be read.

6. Observe for agglutination and/or hemolysis. Record reactions.

7. Add two (2) drops of LISS, mix well and incubate for 15-30 minutes at 37°C. (Albumin incubation is 30-60 minutes.)

8. After incubation, spin all tubes for 15-20 seconds.

9. Observe each tube for agglutination and/or hemolysis. Record reactions.

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 AHG and spin all tubes for 15-20 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 for 15-20 seconds. 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, -P1, -I, -M, -N, and Lu+. 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, s, and Lutheranb (Lub) 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.
Interpretation of Results - Pulling it all together

Name __________________________    Date ___________

Please answer the following questions based on the laboratory results from today. Please attach this page to your laboratory results’ page.

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?
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 at 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 AHG 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 three 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 37°C and/or AHG. (2 points)

13. List the antibody(ies) that 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. (4)

19. If you are having any difficulty with any concepts in Immunohematology, please write your questions or concerns. The questions or concerns will be kept anonymous and discussed with everyone because others may have the same questions.
20-24. (20 points) Refer to the attached panels, numbered 20-24. In each, at least 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. Three points will be awarded for each line in the “Unable to rule out” column when the correct antibodies are listed. (The checkmark in the “Check Cell” (CC) column indicates the results were 1+ to 4+).

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