Exercise 3  

ABO and D Typing

Textbook: Blaney, Chapter 4 The ABO and H Blood Group Systems

Skills: 20 points

Objectives

1. Explain why the ABO blood groups are the most significant of all blood group systems.
2. State the 4 major ABO blood groups.
3. List each blood group and state the ABO antigens present on the red blood cells.
4. List each blood group and state the ABO antibodies expected to be present in the serum/plasma.
5. State what is detected in performing the ABO forward type.
6. State what is detected in performing the ABO reverse type.
7. Given a set of forward and/or reverse typing reactions, interpret the results.
8. Define the term “ABO discrepancy”.
9. List 6 common causes of ABO discrepancies.
10. List 6 methods which may be utilized to resolve ABO discrepancies.
11. State the action to be taken when an ABO discrepancy occurs and patient urgently needs a transfusion.
12. State the significance of the D antigen as it relates to transfusion.
13. Define “immunogenic”.
14. State the percentage of the population which is D positive and D negative.
15. State the principle of the weak D test.
16. State the significance of a weak D positive person as a patient and as a blood donor.
17. List 2 instances, when typing for the D antigen, in which a D control must be run.
18. Compare and contrast the purpose of the D control for an AB pos person versus a D negative person.

Discussion

The ABO typing is the most important test performed in transfusion practice today. The single most common cause of transfusion-related fatalities is due to a patient being transfused with ABO incompatible blood. These reactions occur because individuals form potent, naturally occurring antibodies to ABO red cell antigens which they do not possess. When transfused with ABO incompatible blood, an immediate antigen/antibody reaction occurs which, if not detected in time, may be fatal.

The terms D positive and D negative refer to the presence or absence of the D antigen on the red cell. Approximately 85% of the general population have the D antigen on their red cells. After A and B antigens, the D antigen is the most important antigen in transfusion practice. The D antigen is very immunogenic. Individuals who lack the D antigen must be given D negative blood to prevent antibody stimulation.

Principle

ABO antibodies are of the IgM class and react preferentially at 22°C (RT) or below. Incubation at warm temperatures may cause a false negative reaction. Enhancement of weak reactions may be obtained by RT incubation or incubation at 4°C.
There are three (3) types of tests which must be performed in order to determine an individual’s ABO/D type: ABO forward, ABO reverse and D typing.

1. **ABO Forward Typing**
   
   *This test is used to detect the presence or absence of A and/or B antigens on an individual's red blood cells.*
   
   An individual's ABO group is determined by testing the red blood cells with reagent anti-A and anti-B sera. Agglutination of the individual's red cells by the appropriate antisera signifies the presence of the antigen on the red cell while no agglutination with the antisera signifies its absence.

   Additional testing of the red cells with Anti-A,B sera is used for the detection of weak subgroups of A or B. Because weak A or B subgroups have fewer antigens present on the red blood cells, very weak or negative reactions may occur with anti-A or anti-B serum, but will give stronger or positive reactions with anti-A,B serum.

   In the past Anti-A sera was obtained from group B individuals, anti-B serum was obtained from group A individuals, and anti-A,B serum was obtained from group O individuals. Most facilities currently use monoclonal source antibodies for blood grouping.

2. **ABO Reverse Typing**
   
   *This test is used to detect ABO antibodies in an individual's serum, and is used to confirm the ABO Forward Typing.* There are structures present in nature on certain bacteria and pollens which are very similar to the A and B red cell antigens. Individuals will produce potent, naturally occurring antibodies directed against the ABO antigens they lack. The patient's serum is mixed with reagent group A, cells. Agglutination indicates the presence of Anti-A in the patient's serum. Mixing the patient's serum with reagent group B cells similarly allows for the detection of anti-B in the patient's serum.

   The outcome of the serum typing (Reverse Typing) is compared with the outcome of the cell typing (Forward Typing) to ensure the accurate ABO determination. Any discrepancy must be resolved before final interpretation of the blood group is made.

   *Group A* individuals lack the B antigen and their serum will agglutinate the reagent B cells due to their naturally occurring anti-B. Their serum will not agglutinate the reagent A cells since this antigen is present on their own cells.

   *Group B* individuals lack the A antigen and their serum will agglutinate A cells with their naturally occurring anti-A. Their serum will not agglutinate the reagent B cells.

   *Group O* individuals lack both A and B antigens and their serum will agglutinate both the A and B reagent red cells. *Group O* individuals have 3 naturally occurring antibodies in their serum: anti-A, anti-B and anti-A,B.

   *Group AB* individuals have both A and B antigens on their red cells, and their serum will not agglutinate the A or B reagent red cells.
These two (2) tests, forward and reverse typing, constitute the ABO typing of an individual and are one of the “check” systems in routine Blood Banking. Remember that the forward typing is indicative of the antigens present on an individual's red cells, while the reverse typing is indicative of the antibodies present in the individual's serum. The forward type must correlate with the reverse type and any discrepancy must be resolved.

An ABO discrepancy is a situation in which reactions obtained in the forward type DO NOT match or correlate with reactions obtained in the reverse type. For example, no agglutination when testing a patient with anti-A and anti-B indicates that a patient is group O. The expectation then is that both the A and B reagent cells will be agglutinated by the patient’s serum. If negative reactions occur with one or both reagent red cells then an ABO discrepancy has occurred. No blood may be issued for transfusion until the ABO discrepancy has been resolved. If the need is urgent the technician may need to consult with the patient’s physician and the pathologist. The type of discrepancy involved will determine how the situation is to be handled.

Reasons for an ABO discrepancies:

- subgroup of A (A_2 or A_B) with anti-A_1 - patient forward types as A but agglutinates A_1rbc's.
- strong cold autoagglutinins present - A, B or AB patients will agglutinate reagent rbc’s
- patient may be an infant, elderly or immunodeficient resulting in decreased antibody levels - lack of expected agglutination reactions of reverse cells.
- rouleaux - unexpected positive reactions in reverse cells.
- unexpected antibodies in the serum -unexpected positive reactions in reverse cells.
- patient’s red blood cells heavily coated with protein - unexpected positive reactions in forward type.

Resolution of ABO discrepancies:

1. Repeat the forward and reverse - this should always be the first course of action
2. If patient is group A or AB, but reverse types as an O (false positive), test the RBCs with anti-A_1. If the patient is A_2 or A_B test their serum against A_2 cells. Lack of agglutination of the A_2 cells indicates that the patient has anti-A_1.
3. If the reverse type gives false negative reactions allow the serum and cells to incubate at RT for 5-10 minutes. Respin. False negative reactions are usually due to low levels of antibody being present. The incubation period allows time for additional antibody attachment.
4. False positive reactions in the reverse may be due to strong cold agglutinins additional testing must be performed cold agglutinin titer, prewarmed technique to resolve this problem.
5. False positive results in the reverse may be due to rouleaux. Examine tubes microscopically to confirm. Use saline replacement technique (spin tubes down, remove serum, add saline, respin and read). Negative reactions will be obtained if rouleaux was present.
6. False positive results in the forward type may be due to the red blood cells being so heavily coated with immunoglobulin that the cells spontaneously agglutinate. Perform a DAT, a positive result confirms this as the cause. An extensive work up would be initiated. Transfuse with O negative if this is an emergency or valid typing cannot be determined.

3. **D Typing**

After ABO, the most important antigen in transfusion practice is D. The D antigen is a member of the Rh system. Unlike the ABO system, Rh antibodies are not naturally occurring, therefore, persons who lack the D antigen do not have anti-D antibody in their serum. Antibody formation results from exposure to immunizing red cells that possess the D antigen, either through transfusion or pregnancy.
A high proportion of D negative subjects exposed to the D antigen will produce antibodies. The immunogenicity of D (i.e., the likelihood of its provoking an antibody if introduced into a D-negative recipient) is greater than that of virtually all other red cell antigens studied. Of D negative individuals who receive a single unit of D positive blood, 50-75% can be expected to form anti-D. Exposure to amounts as small as 0.1 ml of red blood cells can cause antibody formation. All D negative recipients must receive D negative blood.

Detecting the D antigen consists of testing the individual's red blood cells with anti-D. An Rh control is necessary if the anti-D reagent used contains a high protein media to enhance the strength of the reaction. Some individual's red cells may spontaneously agglutinate when placed in this high protein media, giving a dangerous false positive reaction. The Rh control consists of the high protein media only. If the control is positive, the test is invalid and alternate methods must be used to determine the individual's true D type. If the D type cannot be determined, the individual must receive D negative blood.

A positive reaction with anti-D and a negative control indicates that the individual possesses the D antigen on their red cell and is D positive. If a negative reaction is obtained in both the anti-D and Rh control tubes, further testing is required to establish D type.

Most clinical blood banks are now using saline anti-D or chemically modified anti-D which does not require a control to be run unless the patient/donor is AB positive. If a person is AB positive there will not be a forward typing tube having a negative reaction. One must ensure the validity of results by running a negative control as specified by the manufacturers instructions. A negative control tube verifies that the individual is AB positive while a positive reaction in the control tube indicates the need for additional testing to determine the individual's ABO and D type.

Not every D positive cell sample reacts with the anti-D sera on immediate spin. The D antigen may actually be present, the cells may actually be D positive, but additional testing is needed to demonstrate the presence of the weakly expressed antigen. This type of reactivity is due to variants of the D antigen collectively called weak D positive (previously called D⁰).

In the D⁰ test, the negative D tube and control tube are allowed to incubate at 37°C for 15 minutes. If the D antigen is present, the cells will be sensitized with the anti-D contained in the D tube. To determine whether this has occurred, the cells are washed and anti-human globulin (an anti-antibody) is added. If the cells have been sensitized, agglutination will occur, indicating that the individual is D positive. The correct term for a patient whose cells react at anti-human globulin phase with anti-D is weak D positive. If negative reactions are obtained, the individual is D negative. The control tube must be negative in order for the test to be valid.

If the weak D test and Rh control tubes are positive a DAT must be performed. If the DAT is positive this indicates the patient cells are coated with immunoglobulin. A false positive D⁰ test may be obtained on individuals whose red blood cells are coated with immunoglobulin.

The weak D type may cause confusion when transfusion is required. Weak D donors are considered D positive. The weak D recipient is considered D negative. The weak D test is not required for recipients by AABB Standards. But due to the confusion of when this test should or should not be performed, it is best to perform the test on all patients and donors.
4. **Significance of the D control**

Many students have a difficult time understanding the significance of the two situations in which a D control must be run. When a patient types as AB positive there is no tube in the forward type with a negative reaction. And even though the reverse type might match (no agglutination of A or B reagent rbc’s) this could be a false negative due to a decreased antibody production in the patient. The positives in the forward tube could be due to spontaneous agglutination of the red blood cells due to heavy protein coating of the red blood cells. If one to were erroneously omit the D control and the patient was in reality an A positive, then transfusion of AB positive would be potentially fatal due to the patients naturally occurring anti-B antibodies. *A negative D control will verify that the patient’s rbc’s were NOT spontaneously agglutinating.*

Alternatively, when a patient types as D negative an additional phase of the test, the weak D test, must be performed to confirm the patient as truly D negative. *The purpose of the D control for the weak D test is to control the anti-human globulin (AHG) portion of the test.* Some patient’s rbc’s may be coated with antibody due to disease (auto-immune hemolytic anemia) or in response to a recent transfusion. If the D control is positive at the AHG phase of testing this invalidates the test and will prompt additional testing of the patient’s rbc’s. If one to were erroneously omit the D control then one would incorrectly interpret the patient as a weak D positive. In an emergency situation in which no D negative blood was available a weak D positive blood may receive D positive blood. The D antigen is very immunogenic. Transfusion with D positive blood may immunize the patient to the D antigen (the patient will produce anti-D). This would be devastating in a female patient who plans to have children.

In summary, the D control for an AB positive is to control the immediate spin phase of testing to ensure that the patient’s rbc’s are not spontaneously agglutinating. The D control for D negative patients is to control the AHG phase of testing and prevent failure to detect a positive reaction due to patient’s rbc’s being coated with antibody. If after studying your text, lecture guide and lab procedure you continue to have difficulty with this concept make an appointment with your instructor.
Models of D and D⁺ cells

- D cells agglutinated by 75 anti-D in albumin enhanced medium.
- D⁺ cells unagglutinated by 75 anti-D in albumin enhanced medium.
- D⁺ cells coated with 75 anti-D agglutinated by 75 anti-human globulin.

- Anti-A + Centrifugation → Antigen A on patient cells → Pellet of agglutinated particles.
Supplies
1. ABO/D typing reagents (in reagent rack)
2. One ABO typing slide for each patient
3. Six 12x75 Test tubes for each patient
4. Plastic Blood Bank Pipets
5. Blood Bank Saline Squeeze Bottle (make sure it is labeled 0.85% NaCl)

A. ABO and D Typing - Tube Test

Procedure

This is the procedure used most frequently for accurate ABO and D determinations and must be used for pre-transfusion testing. CAUTION: NEVER place any specimen or reagents in an unlabeled tube.

1. Write patient's full name and hospital number on TWO tubes, one for serum and one for cell suspension tubes. With a pipette, remove serum from clotted centrifuged specimen (try not to contaminate with cells) and place in one tube. (NOTE: If serum is already separated, check that the Typenex #’s on the serum tube matches the typenex # on the clot tube. Write the patient’s full name and hospital number on it, label an additional tube for patient rbcs and proceed to #3.)

2. Place tip of the pipette at bottom of clotted specimen and aspirate a small amount of cells (approximately 5 drops) and place in your second labeled tube.

3. Fill the tube containing cells three-fourths full with saline, using enough force to thoroughly resuspend the cells. Do NOT overfill. DO NOT place tip of saline bottle into tube.

4. Place serum and cell suspension across from each other (if volumes are the same) in the serofuge head. If the volumes ARE NOT the same you will need to prepare balance tubes. Spin serum and cell suspension one (1) minute in the serofuge.

While serum and cell suspension are spinning label 6 additional tubes with patient initials at the top of each tube and the identity of the reagent to be added below the initials. Do this by placing the palm of your hand over all six tubes with the open ends at the top. You can then quickly label all tubes at one time. Place in rack as indicated below.

<table>
<thead>
<tr>
<th>Clot</th>
<th>Pt RBCs</th>
<th>anti-A</th>
<th>anti-B</th>
<th>anti-A,B</th>
<th>anti-D</th>
<th>Pt Serum</th>
<th>A1 cells</th>
<th>B cells</th>
</tr>
</thead>
</table>
5. Decant the saline from the cell suspension completely by inverting the tube over the waste bucket, allowing all saline to drain out, shake the inverted tube 3 times. DO NOT shake tube while saline is draining out, you will cause the rbcs to be rinsed out of the tube into the waste bucket.

6. Add enough saline to the patient’s cells to obtain a 4-6% cell suspension (compare color with that of the reagent red cells in the pipette).

7. Add one (1) drop of reagent antiserum in the forward typing tubes (anti-A in anti-A tube, anti-B in anti-B tube, etc.)
8. Add three (3) drops patient serum to the A cell and B cell reverse typing tubes.

**NOTE:** Visually inspect all tubes at this time to make certain that all tubes have serum or anti-serum in them.

9. Add one (1) drop of patient cells to each forward typing tube.

10. Add one (1) drop of well mixed reagent reverse cells to each appropriate tube.

11. Mix tubes thoroughly by gently shaking.

12. Spin all tubes for 15 seconds. **NOTE:** Serofuge MUST be balanced.

13. Read and immediately record graded results as they are read.

**NOTE:** The manner in which the RBCs are dislodged from the bottom of the tube is critical. Shake the tube gently until all cells are dislodged. This is most easily accomplished by holding the tube between thumb and forefinger and gently agitating, then tilting the tube. Avoid over shaking which breaks up fragile agglutinates (false negative reaction) while inadequate mixing may result in a false positive interpretation. If you get reactions of less than 3-4+ in the D or reverse typing tubes you are shaking too hard which could result in a false negative result.

14. If anti-D is positive and patient is not Group AB positive, stop here.
   a. If tube containing anti-D is negative, continue to step 15.
   b. If patient appears to be AB pos go to step 23.

15. Set up a tube labeled with patient initials and “D ctrl”. Place one drop of Rh control in the tube followed by 1 drop of the patient’s washed rbc suspension. Spin, read and record the D ctrl tube. Place both the negative D tube and D ctrl tube in the 37° heat block for 15 minutes.

16. After 15 minutes, remove the D and D ctrl tubes, wash three (3) times with saline as follows:
   a. Fill tubes three-fourths full with saline.
   b. Spin in serofuge for one (1) minute.
   c. Completely decant saline by turning upside down and allowing all saline to drain out of the tube, then shake three times. **NOTE:** do NOT shake as the saline is draining out or you will LOSE your rbcs and will need to repeat the test.
   d. Resuspend cell button by vigorous tapping with your finger.
   e. Add saline with sufficient force to resuspend cell button.
   f. Repeat a-e two (2) additional times, for a total of three washes.

17. After decanting saline after last wash continue to hold the tubes upside down and blot ends of tubes with biowipe to obtain a dry cell button.

18. Add two (2) drops of anti-human globulin serum to each tube. **Mix well** and centrifuge for 15 seconds.

19. Gently resuspend cell button, looking for agglutination as in step 15 above.

20. If negative macroscopically, observe microscopically also. **Record results now.**

21. To all tests showing a negative reaction, add one (1) drop of coombs control cells.
22. Centrifuge for 15 seconds. Should get 1 to 3+ reaction. If negative reaction is obtained, test is invalid and must be repeated.

23. If all tubes in the forward type are positive (patient appears to be AB positive) a negative control tube must be run.
   a. Label a tube with patient initials and "D ctrl" below it.
   b. Place 1 drop of the patient red blood cell suspension in the tube.
   c. Add one drop of Rh control reagent to the D ctrl tube, mix well.
   d. Centrifuge for 15 seconds.
   e. Shake gently to resuspend.
   f. If tube is negative, it confirms patient is AB positive, if it is positive additional testing must be done.

Grading Serological Reactions in Tube Testing

Grade Appearance

4+ Complete agglutination – no unagglutinated RBCs
3+a Intermediate between 3+ and 4+
3+ Strong reaction – a few detached masses of agglutinated RBCs; no unagglutinated RBCs
3+w Intermediate between 2+ and 3+
2+a Intermediate between 2+ and 3+
2+ Moderate reaction – large agglutinates in a sea of smaller agglutinates; few unagglutinated RBCs
2+w Intermediate between 1+ and 2+
1+a Intermediate between 1+ and 2+
1+ Weak reaction – many agglutinates of up to 20 RBCs with some smaller agglutinates and unagglutinated RBCs
1+w Intermediate between ± and 1+
± Granular reaction – scattered agglutinates of 6-8 RBCs with many unagglutinated RBCs seen microscopically
0* Rough reaction – RBC button does not disperse smoothly; edge of button appears “rough” (characteristic of some HTLA antibodies)
0 No reaction
MF* Mixed-field strong – large complete agglutinates with a small number of unagglutinated RBCs
MF Mixed-field – moderate-sized agglutinates together with unagglutinated RBCs
MFw Mixed-field weak – few clumps of agglutinated RBCs, but majority of RBC unagglutinated
H* Strong hemolysis – few or no intact RBCs seen
H Moderate hemolysis – some intact RBCs present
Hw Trace hemolysis – many intact RBCs present

### Interpretation

<table>
<thead>
<tr>
<th>Forward</th>
<th>anti-A</th>
<th>anti-B</th>
<th>anti-A,B</th>
<th>Reverse</th>
<th>A cells</th>
<th>B cells</th>
<th>Blood Group Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
<td></td>
<td>+</td>
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<td>O</td>
<td></td>
<td></td>
<td>A,B</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>anti-D</th>
<th>D ctrl</th>
<th>D⁺</th>
<th>D (Du) ctrl</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+</td>
<td>O</td>
<td></td>
<td></td>
<td>D pos</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>3+</td>
<td>O</td>
<td>D pos&lt;sup&gt;∧&lt;/sup&gt;</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>D neg</td>
</tr>
</tbody>
</table>

If the control for the weak D test gives a positive result then a DAT must be performed to ensure valid results. If the DAT is negative, this confirms that the patient is a weak D, if the DAT is positive, additional testing must be performed.

If patient is AB positive, and the D control is positive, the test cannot be interpreted and additional testing must be performed.

**Report results as ABO type followed by D type. Example: A pos: O pos; AB neg**
B. ABO Slide Test

Procedure

Interpretation of Results

Slide tests should be performed according to directions for the specific antiserum used.

1. Place one drop each of anti-A, anti-B and anti-D on separate areas of a properly labeled slide.
2. To each antiserum, add one (1) drop of cells from the washed cell suspension used in procedure A.
3. With a separate clean applicator stick, mix each cell-antiserum mixture thoroughly over the entire circle.
4. Rotate or tile the slide and observe for agglutination for a period not to exceed two (2) minutes.
5. Record results.

<table>
<thead>
<tr>
<th>anti-A</th>
<th>anti-B</th>
<th>ABO group</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>O</td>
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<tr>
<td>+</td>
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<td>+</td>
<td>A,B</td>
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<table>
<thead>
<tr>
<th>anti-D</th>
<th>D control</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Run if patient types as AB positive</td>
<td>D positive</td>
</tr>
<tr>
<td>O</td>
<td>Not necessary</td>
<td>D negative</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Test invalid</td>
</tr>
</tbody>
</table>

Homework

Next week you must turn in an index card with the ABO and D tube test written and condensed on it. Only one index card is needed. This is to be written in a simplified manner to aid you in performing this procedure. THIS IS NOT OPTIONAL. This card will be used throughout your blood bank courses this semester and during your clinical rotation at the hospital.
Exercise 3  ABO and D Typing

Recording Results

For each of the following record the patient name (last name first) and the identification number directly from the tube of blood. Record your reactions and interpretations as explained below.

A. Tube Test

Record graded reactions (0-4+). Leave blank if test was not done. Report “interpretation” as blood group followed by “pos” if D pos, or “neg”, for D neg. Example: O pos

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-AB</th>
<th>Anti-D</th>
<th>*D Control</th>
<th>D Control</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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</table>

*This is performed only if a high protein anti-D reagent is used or the patient appears to be AB positive.

B. ABO Slide Test

Use the cell suspension prepared in procedure A, “Tube Test” for this procedure. The slide test is qualitative, so the results of the reactions with each typing sera is recorded as “pos” or “neg”. For the “Interp” record the blood type followed by “pos” for D positive or “neg” for D negative. Example: A pos

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>anti-A</th>
<th>anti-B</th>
<th>anti-D</th>
<th>Interp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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</tbody>
</table>
Exercise 3  

ABO and D Typing  

Study Questions

1. What is the most common cause of transfusion related fatalities? (1 point)

2. State the immunological reason why ABO typing is the most important test performed in pretransfusion testing. (1 point)

3. Define the terms “D positive” and “D negative”. (1 point)

4. What is being detected in the forward typing test? (0.5)

5. What is being detected in the reverse grouping test? (0.5)
6. State the purpose for using anti-A,B typing sera. (1 point)

7. List the three ABO typing seras AND state the blood group which produces each. (1.5 points)

8. For each of the blood groups listed below state what antibodies will be detected in the serum and fill in the expected reactions (positive or negative) with the A\(^1\) and B cells. (4.5 points)

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Antibodies Present in the Serum</th>
<th>Expected reaction of serum with A(^1) cells</th>
<th>Expected reaction of serum with B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. AB</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. State 4 conditions/reasons which may result in an ABO discrepancy. (2 points)
10. List 4 techniques which may be utilized to resolve ABO discrepancies. (2 points)

11. Define “immunogenicity” as it relates to the D antigen? (1 point)

12. What percentage of the population is D positive? D negative? (1 point)

13. Correctly interpret the following D typing reactions according to the information provided in the laboratory instructions. (1.5 points)

<table>
<thead>
<tr>
<th></th>
<th>Anti-D</th>
<th>D ctrl</th>
<th>D+</th>
<th>D+ ctrl</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>4+</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>B.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C.</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
14. D controls are unnecessary when using saline or chemically modified anti-D *EXCEPT* when the patient is which AB pos or D neg. Explain in detail why a control must be run in these situations. (2.0 points)
   a) AB post
   b) D neg

15. State the purpose of performing the weak D test. (1 point)

16. Describe the transfusion status (D interpretation) of the weak D individual as a donor and as a recipient. (1 point)

17. The weak D test is positive. What test must be performed to ensure that this is not a false positive result? (0.5)
18. According to the reporting format demonstrated, interpret the following ABO/D types. Notice that you are to write your interpretation of the forward type in the “Interp Forward” column, the reverse type in the “Interp Reverse” column, then compare the 2 determine validity of the results. If the reaction given are NOT valid write “invalid” for that portion of your interpretation. Once you have compared the results write “yes” for valid (forward and reverse match) or “no” for invalid (forward and reverse did not match). (2 points each)

<table>
<thead>
<tr>
<th>Forward Typing</th>
<th>Interp Forward</th>
<th>Reverse Typing</th>
<th>Interp Reverse</th>
<th>Valid?</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-A</td>
<td>anti-B</td>
<td>anti-A,B</td>
<td>anti-D</td>
<td>D control</td>
</tr>
<tr>
<td>a. O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>NA</td>
</tr>
<tr>
<td>b. O</td>
<td>O</td>
<td>1+</td>
<td>3+</td>
<td>NA</td>
</tr>
<tr>
<td>c. O</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>NA</td>
</tr>
<tr>
<td>d. 4+</td>
<td>4+</td>
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<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>e. 4+</td>
<td>4+</td>
<td>4+</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>f. 3+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>O</td>
</tr>
</tbody>
</table>

If you answered “No” in the “Valid?” column describe the discrepancy and use the information provided in the laboratory to provide an explanation. Extra credit awarded for correct problem solving.