Exercise 3  ABO and D Typing

Textbook:  Blaney, Chapter 4 The ABO and H Blood Group Systems and 5 Rh Blood Group System

Skills: 20 points

Objectives

1. State the reason why ABO blood groups are the most significant of all blood group systems.
2. List the 4 major ABO blood groups and state the antigens present on the RBCs and antibodies present in sera.
3. State what is being detected when performing the ABO forward type.
4. State what is being detected when performing the ABO reverse type.
5. List each blood grouping reagent and state what it is used for.
6. Given a set of forward and/or reverse typing reactions, interpret the results.
7. Define the term “ABO discrepancy.”
8. Describe four problems associated with ABO typing discrepancies.
9. List methods which may be utilized to resolve the four problems associated with ABO discrepancies.
10. State the action to be taken when an ABO discrepancy occurs and patient urgently needs a transfusion.
11. State the significance of the D antigen as it relates to transfusion.
12. Define “immunogenic.”
13. State the percentage of the population which is D positive and D negative.
15. State the significance of a weak D positive person as a patient and as a blood donor.
16. List 2 instances, when typing for the D antigen, in which a D control must be run.
17. Compare and contrast the purpose of the D control for an AB positive person versus a D negative person.
18. Perform an ABO/D type on two patient samples with 95% accuracy.

Discussion

The ABO typing is the most important test performed in transfusion practice today. The most common cause of life-threatening hemolytic transfusion reactions 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. These antibodies have the ability to fix complement and cause intravascular hemolysis of incompatible RBCs. When transfused with ABO incompatible blood, an immediate antigen/antibody reaction occurs which, if not detected in time, may be fatal. Because of the dire consequences associated with ABO incompatible transfusions, ABO typing and compatibility testing remain the foundation of all pre-transfusion testing.

The terms D positive and D negative refer to the presence or absence of the D antigen on the RBC. Approximately 85% of the general population has the D antigen on their RBCs. After A and B antigens, the D antigen is the most important antigen in transfusion practice. The D antigen is highly immunogenic. Individuals who lack the D antigen may form potent immune antibodies to the D antigen and must be given D negative blood to prevent antibody stimulation.

Principle

The ABO system contains four major blood phenotypes: A, B, AB and O. ABO/D testing is designed to detect the presence or absence of antigens on the RBCs and antibodies in the serum or plasma. ABO antibodies in the serum or plasma of group A and group B individuals are predominantly of the IgM class; while anti-A, anti-B, and anti A,B from group O individuals are predominantly of the IgG class. Both immunoglobulin classes of ABO antibodies react preferentially at room temperature (RT) 20-24°C or below. Incubation at warm temperatures may cause a false negative reaction due to dissociation of the antibody from the RBC. Enhancement of weak reactions may be obtained by extending RT incubation or incubation at 4°C.
An inverse relationship exists between the presence of A and B antigens on RBCs and the presence of anti-A, anti-B or both in serum or plasma. An individual who lacks the A antigen on the RBCs will have potent anti-A in their serum or plasma.

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. ABO typing requires testing of RBCs for A and B antigens and testing serum or plasma for the presence of anti-A and anti-B antibodies. A third component of the test is to determine the presence or absence of the D antigen.

1. ABO Forward Typing

This test is used to detect the presence or absence of A and/or B ANTIGENS on an individual’s RBCs. An individual’s ABO group is determined by testing the RBCs with reagent anti-A and anti-B sera. Agglutination of the individual’s RBCs by the appropriate antisera signifies the presence of the antigen on RBC. No agglutination with the antisera indicates the absence of the corresponding antigen.

Additional testing of the RBCs with Anti A,B may be used for the detection of weak subgroups of A or B. Because weak A or B subgroups have fewer antigens present on the RBCs, very weak or negative reactions might occur with the anti-A or anti-B reagent, but will give stronger or positive reactions with the anti-A,B. The use of potent monoclonal antibodies has dramatically decreased the use of anti-A,B and it is no longer routinely used.

In the past, anti-A sera was obtained from group B individuals, anti-B sera from group A, 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 or plasma, and is used to confirm the ABO Forward Type. There are structures present in nature on certain bacteria and pollens which are very similar to the A and B antigens on RBCs. Individuals will produce potent, naturally occurring antibodies directed against the ABO antigens they lack. The terms “non-red cell stimulated antibodies” and “isohemagglutinins” are also used to describe these antibodies. The patient’s serum is mixed with reagent group A1 cells. Agglutination indicates the presence of anti-A in the patient’s serum. Mixing the patient’s serum with the 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 anti-B present in their serum. Their serum will NOT agglutinate reagent A1 cells since the A1 antigen is present on their own cells.

- Group B individuals LACK the A antigen and their serum will agglutinate the reagent A cells due to anti-A present in their serum. Group B individual’s serum will NOT agglutinate reagent B cells.

- Group O individuals LACK both A and B antigens. Their serum will agglutinate both reagent A and reagent B cells. Group O individuals have 3 naturally occurring antibodies: anti-A, anti-B, and anti-A,B.

- Group AB individuals HAVE both A and B antigens. Their serum will NOT agglutinate reagent A or reagent B cells. Group AB individuals do not have any naturally occurring antibodies in their serum.

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 the individual’s RBCs, while the reverse typing is indicative of the antibodies present in the individual’s serum or plasma. The forward type must correlate with the reverse type. Any discrepancies must be resolved before a final determination is made.
Helpful hint: Think about what is “known” in the test. If you are adding known antibodies (typing sera) to unknown RBCs you are detecting antigens on the RBCs. If you are adding unknown serum or plasma to RBCs of known antigenic make up you are detecting antibodies in the serum or plasma.

<table>
<thead>
<tr>
<th>Blood type (genotype)</th>
<th>Type A (AA, A1)</th>
<th>Type B (BB, B1)</th>
<th>Type AB (AB)</th>
<th>Type O (ii)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cell Surface Antigens (Phenotype)</td>
<td><img src="image" alt="A antigen" /></td>
<td><img src="image" alt="B antigen" /></td>
<td><img src="image" alt="AB antigen" /></td>
<td><img src="image" alt="O antigen" /></td>
</tr>
<tr>
<td>Plasma Antibodies (Phenotype)</td>
<td><img src="image" alt="A antibody" /></td>
<td><img src="image" alt="B antibody" /></td>
<td><img src="image" alt="D antibody" /></td>
<td><img src="image" alt="O antibody" /></td>
</tr>
</tbody>
</table>

- Blue reagent = anti-A which has agglutinated the RBCs, indicates the presence of the A antigen.
- Yellow reagent = anti-B, NO agglutination presence indicates B antigen is not present.
- Clear reagent = anti-D which has agglutinated the RBCs, indicates D antigen is present.
- Interpretation is A positive.

ABO Discrepancies
An ABO discrepancy is a situation in which reactions obtained in the forward type DO NOT correlate with reactions obtained in the reverse type. There are two types of reactions, unexpected positives and unexpected negatives. For example, no agglutination of the patient RBCs with anti-A and anti-B indicates the patient is group O. The expectation then is that both the A and B reagent cells will be agglutinated by the patient’s serum due to the expectation of anti-A and anti-B being present in the patient’s serum or plasma. If negative reactions occur with either reagent cell, then an ABO discrepancy has occurred. No blood may be transfused until the ABO discrepancy has been resolved. If the need for blood products 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 should be handled. In most situations group O blood may be transfused while the discrepancy is being investigated.
ABO Discrepancies – Causes and Resolutions

The FIRST course of action to resolve an ABO discrepancy is to REPEAT the forward and reverse type. If the same discrepant results are obtained it must be determined whether the problem lies with the RBCs or the serum.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Causes and Factors to Consider</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Unexpected positive in reverse type.</td>
<td>Forward types as Group A but reaction, usually weak, with A₁ reverse cells. Subgroup of A with anti-A₁</td>
<td>Test patient cells with anti-A₁ lectin should be negative. Test patient serum or plasma with A₂ cells should be negative. Strong cold auto-agglutinin</td>
</tr>
<tr>
<td>2. Unexpected negative in reverse type.</td>
<td>Low antibody level due to being elderly, an infant or immunocompromised.</td>
<td>Incubate reverse at RT for 15-30 minutes, respin. If still negative incubate at 4°C for 15-30 minutes with an autocontrol.</td>
</tr>
<tr>
<td>3. Unexpected positive in forward type.</td>
<td>Patient RBCs heavily coated with protein.</td>
<td>Perform Direct Antiglobulin Test – will be discussed later in the course.</td>
</tr>
<tr>
<td>4. Unexpected negative in forward type.</td>
<td>Weak subgroup of A or B.</td>
<td>Incubate at RT for 15-30 minutes. May have to do higher level testing which will be discussed later in this course.</td>
</tr>
</tbody>
</table>

NOTE: This chart illustrates the most common causes, there are many other situations which may occur which will be covered later in this course.
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 stimulated by non-red cell antigens such as bacteria and plants; therefore, persons who lack the D antigen do not have anti-D antibody in their serum or plasma. Antibody formation results from exposure to immunizing red cells that possess the D antigen, either through transfusion or pregnancy. Approximately 85% of the population is D positive.

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 D-positive RBCs can cause antibody formation. All D negative recipients must receive D negative blood.

Detecting the D antigen consists of testing the individual’s RBCs with anti-D reagent. A D (Rh) control is necessary if the anti-D reagent uses high protein media to enhance the strength of the reaction. (Reagent choice differs by institution.) Some individual’s RBCs will spontaneously agglutinate when placed in high protein media which yields dangerous false positive reactions. The Rh control consists of the high protein media (no antibody.) 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 products (RBCs or platelets).

A positive reaction with anti-D and a negative control indicates the individual possesses the D antigen on their red cells 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 a D type.

Most clinical blood banks are not using the high protein anti-D mentioned. Most blood banks are using saline anti-D or chemically modified anti-D which does not require a control to be run UNLESS the patient or donor is AB positive. If a person is AB positive there is not a forward typing tube with a negative reaction. One must ensure the validity of results by running a negative control as specified by manufacturer’s instructions. A negative control tube verifies that the individual is AB positive rather than polyagglutinable (agglutinates everything.) A positive Rh control indicates the need for additional testing to determine the individuals ABO and D type.

Weak D Testing

Not every D positive cell reacts with the anti-D reagent sera on immediate spin. It is possible that D antigens are present, but weakly expressed on the red cell. 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 D).

In the weak D test, the negative D tube and Rh control tube are allowed to incubate at 37°C for 15 minutes. If the D antigen is present, the cells will be sensitized (coated) with the anti-D in the reagent serum. To determine whether or not this has occurred, the cells are washed thoroughly and anti-human globulin or AHG (an ANTI-antibody) is added. If the cells have been sensitized, agglutination will occur. This positive reaction indicates that the individual is weak D positive. If negative reactions are obtained, the individual’s type has been confirmed D negative. The Rh control must be negative for any result to be valid. If the D control is positive at the AHG phase of testing, the results of the weak D testing are invalidated. More testing will be required.

If the weak D test and Rh control are positive, a DAT must be performed. If the DAT is positive, this indicates that the patient’s cells were coated with immunoglobulin in vivo. A false positive weak D test may be obtained on individuals whose RBCs are coated with antibodies. The DAT will be discussed in detail in a future laboratory.
The weak D type may cause confusion when transfusion is required. *Weak D donors are considered D positive.* A *weak D recipient is considered D negative.* The weak D test is not required for recipients by AABB Standards. In this course the weak D test will be performed on all patients who appear to be D negative on IS so competence can be achieved performing this test. Weak D testing will not be performed on donors.

### Illustration of normal and weak D antigens

![Illustration of normal and weak D antigens](image)

**Normal D antigen is a mosaic of several parts (cognates)**

**Abnormal D antigen (Weak D) is missing one or more cognates**

### 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. The following information may be helpful.

When a patient types as **AB positive** there is **NO** tube in the forward type with a negative reaction. Even though the reverse type might match (no agglutination of A and B reagent RBCs) this could be a false negative reverse type due to decreased antibody production in the patient. The positive reaction in the forward tube could be due to spontaneous agglutination of the RBCs due to heavy protein coating of the patient cells. If the D control was erroneously omitted and the patient was, in reality an A or B, then transfusion of AB positive would be potentially fatal due to the patient’s naturally occurring anti-B antibodies. A negative D control will verify that the patient RBCs are NOT spontaneously agglutinating. *The purpose of the negative control for an AB positive is to control the IS phase of testing.*

When a patient types as **D negative** the weak D test should be performed to confirm the patient is truly D negative. The patient’s RBCs may be coated with antibody due to disease (auto-immune hemolytic anemia) or an immune reaction to recently transfused RBCs. *The purpose of the D control for the weak D test is to control the anti-human globulin (AHG) portion of the test.*
**Procedure**

**Supplies**

1. Blood bank reagents
2. 12 X 75 tubes
3. Plastic transfer pipettes
4. Blood bank saline squeeze bottle (make sure it is labeled 0.85% NaCl)
5. Biowipes
6. Wooden sticks

**ABO and D Typing—Tube Test**

**Caution: NEVER place any specimen or reagent in unlabeled tube**

1. Write patient’s **FULL name and hospital (MR) number on TWO (2) tubes**, one for serum/plasma and one for RBC suspension. (Note: If serum/plasma is already separated, check that the correct patient’s full name and hospital number are on it, and label and additional tube for the patient’s RBC suspension and proceed to step 4)

2. With a pipette, remove serum or plasma from centrifuged specimen (try not to contaminate with cells) and dispense in one of the labeled tubes. **NOTE: If serum or plasma is already separated, write the patient’s full name and hospital number on it, label an additional tube for patient RBCs and proceed to step 4**.

3. Place the tip of the pipette at the bottom of the clotted specimen or top of cells in an EDTA specimen and aspirate a small amount of red cells (approximately 3-5 drops) and place in the second labeled tube.

4. Fill the tube containing the red blood cells three-fourths (3/4) full of saline, using enough force to thoroughly resuspend the cells. **DO NOT OVERFILL and DO NOT place the tip of the saline bottle into the tube. (You are washing the cells)**

5. Spin the serum and cell suspension in the serofuge for 1 minute. (Make sure the serofuge is balanced. If the serum and RBC suspension are the same volume, then they can be across from each other in the serofuge, if not, prepare balance tubes.)

6. While the serum and RBC suspensions are spining, label eight (8) additional tubes with patient initials at the TOP of each tube and the identity of the reagent below initials. Do this by placing the palm of your hand over all tubes with the open end at the top. You can then quickly label all tubes at once. The “-“ signifies “anti-“ which is followed by the typing sera used. The “R” signifies “retype”. Place in the rack AS INDICATED BELOW:

<table>
<thead>
<tr>
<th>Sample</th>
<th>R-A</th>
<th>R-B</th>
<th>R-D</th>
<th>(space)</th>
<th>Patient Serum</th>
<th>A₁ cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient RBCs</td>
<td>-A</td>
<td>-B</td>
<td>-D</td>
<td>(space)</td>
<td>Patient Serum</td>
<td>A₁ cells</td>
<td>B cells</td>
</tr>
</tbody>
</table>

7. 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 the tube while saline is draining out because you will lose all of the RBCs in the waste bucket**.

8. Add enough saline to the patient’s cells to obtain a 3-5% cell suspension (compare the color with that of the reagent cells in the dropper).

9. Add one (1) drop of reagent antiserum in the forward and D typing tubes.

10. Add three (3) drops patient serum/plasma to the A₁ 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 (reagent) in them.

11. Add one (1) drop of 3-5% patient washed cell suspension to each forward and D typing tube.
12. Add one (1) drop of well mixed reagent reverse cells (A₁ and B) to each appropriate tube.
13. Mix tubes thoroughly by gentle shaking.
14. Spin the forward and reverse tubes for 15 to 20 seconds. Note: The serofuge must be balanced.
15. Read macroscopically and immediately record GRADED reaction, using the chart provided, as they are read. You must hold the tube in your hand in front of the column you will record the results in, observe the reaction and record it. DO NOT place the tube in the rack until you have recorded the results!

NOTE: The manner in which RBCs are dislodged from the bottom of the tube is critical. Shake the tube gently until all cells are dislodged from the bottom of the tube. 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.

Retyping the patient specimen

16. For retyping the patient:
   a. Add one tiny drop of RBCs from original patient sample to retype tubes.
   b. Spin retype tubes for 15 to 20 seconds. Record reactions in “retype” row. NOTE: The forward reactions for the washed cell suspension and retype MUST match with regards to positive and negative. The retype reactions may be weaker due to too heavy a cell suspension but as long as the positive and negative reactions match this is fine.
   c. OR you may use three wooden sticks and place them in the EDTA tube of the patient. Swirl the sticks in the EDTA tubes and wipe excess off of the sticks against the side of the tube as they are removed. Place sticks, one a time, into each of the labeled retype tubes. Swirl the sticks in each tube and remove. Spin the retype tubes for 15 to 20 seconds and record as above. Your instructor may demonstrate the stick method for you, if you have any questions.

17. D CONTROL - If anti-D tube is negative OR all tubes in the forward type are positive (patient appears to be AB positive) a negative D (Rh) control tube must be run.
   a. Label a tube with patient initials and “D ctrl” below initials. 
   b. Place 1 drop of patient RBC suspension in the tube 
   c. Add 1 drop of Rh control reagent to the labeled tube and mix 
   d. Serofuge for 15-20 seconds 
   e. Shake gently to resuspend 
   f. AB Positive - If tube is negative, it confirms the patient is AB Pos; however, if agglutination is observed, further testing is required before a blood type can be determined. STOP HERE for AB Pos. 
   g. D Negative – continue with Weak D test.

18. Weak D test – only performed for D negative patients
   a. Place both the negative anti-D reagent tube you previously read and the D ctrl tube in the 37°C heat block for 15 minutes.
   b. After 15 minutes, remove the D and D ctrl tubes, wash three (3) times with saline as follows:
      1) Place tubes in serofuge and place a rubber band around the tubes.
      2) Fill tubes ¾ full with saline
3) Spin in serofuge for one (1) minute.
4) Completely decant saline by turning the tube upside down and allowing all of the saline to drain out of the tube, then shake 3 times. **NOTE:** Do not shake while the saline is draining or you will wash out all of your RBCs and leave nothing to grade.
5) Resuspend cell button by flicking the tubes as demonstrated by instructor.
6) Add saline with sufficient force to resuspend the cell button. **NOTE:** If after resuspending cells with saline you notice a cell button at the bottom add an additional wash.
7) Repeat steps 2-5 a two (2) more times, for a total of 3 washes.

c. After decanting all of the saline after the final wash, **continue to hold the tubes upside and blot the ends of the tubes** with a biowipe to obtain a dry cell button.

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

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

f. If negative macroscopically observe microscopically and record graded reactions.

g. To all tests that show a negative reaction at the AHG phase, add one (1) drop of Coombs control cells (√ cells.)

h. Centrifuge for 15 seconds. You should get a 1+-3+ reaction with the √ cells. Record results immediately. If a negative reaction is obtained, the AHG reagent was neutralized due to incomplete washing of unbound immunoglobulin, and the Weak D test must be repeated from the beginning.

**Grading Serological Reactions in Tube Testing**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gently shake or tilt the tube to resuspend the red cell button in the tube. The tilt technique uses the solution meniscus to gently dislodge the red cell button from the wall of the tube.</td>
</tr>
<tr>
<td>2</td>
<td>Observe the way that cells are dispersed from the red cell button.</td>
</tr>
<tr>
<td>3</td>
<td>Record reactivity by comparing the agglutinates to the descriptions in the table below. The reactivity should be assessed when the red cells have been completely resuspended from the button.</td>
</tr>
</tbody>
</table>
Grade | Macroscopic Observations
--- | ---
4+ | One solid agglutinate
3+ | Several large agglutinates
2+ | Medium sized agglutinates, clear background
1+ | Small agglutinates, turbid background
1+,w | Very small agglutination, turbid background
w+ or +/- | Barely visible agglutination, turbid background
0 | Negative - no agglutination
MF | Mixed Field – mixture of agglutinated and unagglutinated red cells
H | Complete hemolysis, no RBCs present
PH | Partial hemolysis, but some RBCs remain.

*AABB Technical Manual, 18th edition, 2014*

**Interpretation**

**IMPORTANT:** Do NOT use “+” as your interpretation of your agglutination reaction results. In the following chart “+” simply indicates a positive reaction for agglutination, record your GRADED reactions according to the table on the previous page.

<table>
<thead>
<tr>
<th>Forward Type</th>
<th>Reverse Type</th>
<th>Blood Group Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Optional anti-A,B</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D Typing</th>
<th>Weak D Test</th>
<th>Interpretation of D Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D</td>
<td>Rh ctrl</td>
<td>Weak D</td>
</tr>
<tr>
<td>3+</td>
<td>3+</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>3+</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>3+</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>3+</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*If the D control is positive, the D type is invalid and additional testing must be done.

**REPORT RESULTS** as the ABO type followed by the interpretation of the D type.

**EXAMPLES:**
A Pos
O Pos*
AB Neg
Notes

1. For uniformity and reproducibility, the grading of agglutination reactions should be standardized among all members of the laboratory staff.
2. The grading system should be described in a written procedure available to all staff.
3. The grading system above is not necessarily applicable to column agglutination and solid-phase technologies. For appropriate grading of reactions in newer technologies, the package insert should be consulted.
Exercise 3 ABO and D Typing

Study Questions

Your grade: _______/52 Points

These are due the next class period.

1. State the most common cause of transfusion related fatalities due to immune hemolysis. (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”. (2 points)

4. What is being detected in the forward type? (1 point)

5. What is being detected in the reverse type? (1 point)

6. State the purpose for using reagent anti-A,B. (1 point)

7. List the three (3) ABO typing sera AND state the blood group which produces each. (3 points)

<table>
<thead>
<tr>
<th>Anti-Sera</th>
<th>Blood Group which Produces this Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td></td>
</tr>
<tr>
<td>c.</td>
<td></td>
</tr>
</tbody>
</table>

8. What is the FIRST course of action one must take when an ABO discrepancy is obtained? (1 point)
9. For each of the blood groups listed below state the antibodies which will be detected in the serum or plasma and the expected reactions, positive or negative, with A₁ and B reagent cells. (7 points)

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Antibodies Present in serum or plasma</th>
<th>Expected Reaction with reagent A₁ cells.</th>
<th>Expected reaction with reagent B cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. O</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>

10. List four (4) problems associated with ABO discrepancies. (4 points)

   a. 
   b. 
   c. 
   d. 

11. List one potential CAUSE for each of the four problems associated with ABO discrepancies. (4 points)

   a. 
   b. 
   c. 
   d. 

12. List one potential RESOLUTION for each of the four problems associated with ABO discrepancies. (4 points)

   a. 
   b. 
   c. 
   d.
13. Define “immunogenicity” as it relates to the D antigen. (1 point)

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

15. The D control is unnecessary when using saline or chemically modified anti-D **EXCEPT** when the patient is AB Positive or D Negative. Explain in detail why a control must be run in these situations including why it is necessary and what phase of testing is being controlled. (4 points)
   a. AB Positive
   b. D negative

16. State the purpose for performing the weak D test. (1 point)

17. Describe the transfusion status (D interpretation) of the weak D individual as a DONOR and as a RECIPIENT. (2 points)

18. What blood group may be transfused when there is not time to resolve an ABO discrepancy? (1 point)
19. Interpret the following ABO/D reactions. Follow the instructions for filling in the interpretation table.

<table>
<thead>
<tr>
<th>Forward Type</th>
<th>D Type</th>
<th>Reverse Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-D</td>
</tr>
<tr>
<td>a.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>b.</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>c.</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>d.</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>e.</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>f.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>g.</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

**Instructions for filling in table:** Interpret EACH test separately in the appropriate column and indicate whether that particular result appears valid by writing “yes” or “no” in the “Valid?” column. If an ABO result appears invalid state whether the problem is a potential false positive or negative and whether the problem appears to be with the forward, reverse, or both. If the D type is invalid state the D type of blood to transfuse. (12 points)
Exercise 3 ABO and D Typing

Recording Results

For each of the following, record the patient name (Last name, First name) and the ID number directly from the tube of blood.

1. Record GRADED reactions (0-4+) in EACH column by observing the reaction in the tube while the tube IS IN YOUR HAND.

2. Leave space BLANK if the test was not performed.

3. 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>ID Number</th>
<th>Forward Type</th>
<th>D ** Weak D Test</th>
<th>Reverse Type</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>anti-A</td>
<td>anti-B</td>
<td>anti-D</td>
<td>*D ctrl</td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retype patient 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retype patient 2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Only perform D ctrl if using high protein anti-D reagent OR if the patient appears AB positive.

** Only perform Weak D testing if the patient appears D Negative.