

ABO and Rh Blood Typing

INTRODUCTION

The most commonly used immunohematology test is the ABO and Rh screen. ABO and Rh screening determines the blood type of the patient and can also be used to decide if a pregnant woman with Rh negative blood will need to receive Rh immune globulin (i.e., RhoGam). Rh immune globulin injection helps prevent the mother from forming antibodies against the Rh antigen, a condition that may lead to hemolytic disease of the newborn.

The results of ABO/Rh testing are critical to patient care. If a blood type is incorrectly interpreted, the patient may not receive the proper treatment. The following information provides the essentials of ABO/Rh testing and appropriate quality control (QC) procedures. However, it is not intended as a substitute for adequate training in ABO/Rh testing.

SPECIMEN COLLECTION

ABO and Rh testing require both serum and red blood cells; therefore, the specimen should be collected in a vacuum tube with no preservative (red topped tube).

EDTA tubes (lavender topped) may also be used. If using a red-topped tube, DO NOT USE GEL SEPARATOR TUBES. The gel separator tube will put a layer of gel between the red blood cells and the serum when centrifuged, making it very difficult to obtain the red blood cells. The gel may also become mixed with the red blood cells, causing a negative reading to look positive.

Test the specimen as soon as possible. If there is a delay in testing, properly store the specimen in a refrigerator.

SPECIMEN PREPARATION

Test Tube Method - For red-top tubes, after collection, allow the blood to stand until a clot forms, then centrifuge the specimen to separate the serum from the red blood cells. After centrifugation, store the serum in a separate test tube and retain the red blood cells in the original tube. Be sure to label all tubes with proper patient information to avoid confusing them with other patient samples. For purple-top tubes, centrifuge the specimen to separate the plasma from the red blood cells. The plasma may be stored in a

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DEFINITIONS

Antibody - A substance present in serum that is produced as an immune response to a foreign substance introduced to the body (antigen).

Antigen - A foreign substance that can produce an immune response (antibody).

Agglutination - Clumping of particles (red blood cells)

Antihuman globulin - An antibody to human globulin.

Blood type and Rh factor - Blood type is an individual's blood group characterized as either A, B, O, or AB. Rh is the inheritable antigen measured as either positive or negative. These antigens are present on the surface of the individual's red blood cells.

Coombs check cells - Red blood cells that are coated with immunoglobulin G (IgG) antibody.

Cell suspension - Red blood cells from a patient that are mixed with saline to obtain a 2 to 5 percent solution (cherry color).

D control (Rh control) - Bovine albumin, saline or media defined by the reagent manufacturer, which is used as a negative control for Rh and Weak D (Du) testing.

Weak D (Du) testing - Testing that is done to detect a weak Rh type.

Forward typing - A blood typing procedure whereby patient red blood cells are mixed with Anti-A and Anti-B reagents.

Rh Immune globulin - Concentrated solution of Anti-D from human plasma which is given to pregnant women who are Rh negative and who may be carrying and/or have delivered an Rh positive baby. There are several brands available, e.g., *RhoGam*.

Immunoglobulin - Proteins with known immune response activity.

Plasma - The fluid remaining in separated anticoagulated tubes that contains clotting factors.

Reverse typing - A blood typing procedure where patient serum is mixed with reagent A cells and reagent B cells. The results should be the opposite of forward typing.

Serum - The straw-colored fluid remaining when blood is clotted. Serum does not contain any clotting factors.

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separate labeled tube like the red-topped specimen, or kept in the original tube to reduce pre-analytical error.

After separation, prepare a 2 to 5 percent red blood cell suspension for testing. There are several methods that can be used for creating a cell suspension preparation. Each laboratory should establish its own guidelines for the preparation of cell suspensions. Some suggested procedures include:

1. Using a disposable pipette, transfer approximately 0.2 ml (two drops) of the patients packed red blood cells to a 10 x 75 mm test tube.
2. Fill the tube with saline. Cover and mix.
3. Centrifuge the tube at 3,400 rpm for 30 seconds, then decant the saline.
4. Visually estimate a 2 to 5 percent suspension. Adding approximately 1 ml of saline will make a 3 percent suspension, adding approximately 0.5 ml of saline will make a 5 percent suspension.

Alternative Method For Preparation Of 2 Percent Suspension

1. Place 1 to 2 ml of blood in large tube.
2. Fill with saline and centrifuge.
3. Aspirate/decant the remaining saline away from the red blood cells.
4. Repeat washing (steps two and three) until supernatant is clear. The remaining red blood cells are called a packed red blood cell button.
5. Pipette 10 ml of saline into a clean test tube.
6. Add 0.2 ml (two drops) of the packed red blood cell button to the 10 ml of saline. After the red blood cells settle to the bottom of the test tube, rinse the pipette with the surface layer of the saline to remove any red blood cell residue.

Alternative Method For Preparation Of 5 Percent Suspension

1. Follow steps one to five from the 2 percent preparation.

2. Add 0.5 ml of packed red cell button to the 10 ml of saline and rinse pipette with the saline.

Note: For all methods of preparing a cell suspension, you can use your reverse grouping cells as a visual check on the concentration of your suspension, as the concentration of the reagent red cells range from 2 to 5 percent, as determined by the manufacturer.

TESTING

ABO/Rh testing entails putting a sample of the cell suspension in various reagents, centrifuging at a specific speed for a specific period of time, and then gently shaking the tube. If there is any clumping of blood cells, known as agglutination, the result for that reagent is positive.

Follow the manufacturer's instructions with each reagent. Also, when testing ABO/Rh controls or specimen samples for ABO/Rh, the length of time the tubes are spun, as well as the speed of the centrifuge are important. If the tubes are spun for too long or too fast the red blood cell button may be too difficult to dislodge from the test tube. Use gentle agitation when resuspending the red blood cell button from the tube. If the tube is agitated too hard, a weak positive result can be missed.

ABO Testing

ABO testing should include both forward and reverse typing. Reverse typing is a cross-check for forward typing. However, reverse typing is not recommended when typing newborns and infants under the age of 4 months since they have not developed the proper antibodies necessary for the test to be accurate.

Forward typing uses the patient's *red blood cells*. All red blood cells contain antigens that are specific to the patient's blood type. When antibody A (Anti-A) or antibody B (Anti-B) reagent is added to the patient's red blood cells the antigens on the cells will cause the cells to react with the antibodies. For example, if the patient has blood type A, the patient's red blood cells will clump with antibody A (Anti-A), but will not show any clumping with antibody B (Anti-B). If the patient has blood type B, the patient's red blood cells will clump with antibody B (Anti-B), and will not clump with antibody A (Anti-A). *The forward typing results are considered the patient blood type.*

Reverse typing uses the patient's *serum*. The serum contains antibodies that react with the reagent red blood cells which are coated with antigen A (A cells) or antigen B (B cells). The type of antibodies present in the patient's serum will determine which reagent red blood cells will cause agglutination, and will therefore confirm the blood type. For example, if the reagent A cells are negative (no agglutination), the patient does not have the antibody A in their serum, so the blood type is A. The results from the reverse typing will be the opposite of the forward typing. *Reverse typing results are not considered to be the blood type, but a confirmation of the forward typing results.*

Blood Group Chart

When recording results, a plus sign (+) is considered positive, a zero (0) is considered negative, and all reactions are graded on a scale of zero (negative) to most positively reactive (4+). For example, if the patient is blood type A, then the forward results will have a 3 to 4 plus positive result with the Anti-A and a negative result with the Anti-B. The reverse typing should be the opposite of the forward typing. A cells will be negative and the B cells will be 2 to 4 plus positive. Refer to the chart below for the reactions of each blood group.

Table 1
Blood Group Chart

	Forward Typing		Reverse Typing	
	Anti-A	Anti-B	A Cells	B Cells
Group A	3-4+	0	0	2-4+
Group B	0	3-4+	2-4+	0
Group AB	3-4+	3-4+	0	0
Group O	0	0	2-4+	2-4+

Many clinical conditions, including technical errors, can cause discrepancies between forward and reverse typing. To rule out technical error, repeat all ABO/Rh tests. Also, check the specimen for any small clots; these will frequently give false positive results.

If the reverse typing does not agree with the forward typing, let the reverse typing reaction tube stand at room temperature for 5 to 15 minutes. This incubation period will enhance the reaction between the cells and serum. If a discrepancy remains, further

testing must be done by the laboratory or a reference laboratory. It is imperative that all discrepancies be resolved before reporting the patient's blood type.

Rh Testing

All patients are either Rh D positive or Rh D negative. Testing for the Rh D factor is done by using reagent Anti-D and patient red blood cells. If the patient's red blood cells contain the D antigen (Rh D factor), there will be agglutination when tested with Antibody D (Anti-D) and the patient is considered Rh D positive. If there is no agglutination of cells, then the patient is usually Rh D negative. See next section on Weak D (Du) testing.

With each patient test, a patient control (Rh control) may be indicated depending on the recommendation of the reagent manufacturer. The patient Rh control consists of patient red blood cells and a control substance. This control substance could be albumin, saline or other material defined by the manufacturer. If both the patient control and patient test are positive, the result is considered invalid and should be repeated with washed patient red blood cells. A positive patient control demonstrating agglutination indicates that another substance is present and reacting with the control portion of the Anti-D reagent and not the Anti-D. Don't forget that all prenatal specimens that test negative for Rh (D) should also be tested for Weak D (Du).

Weak D Testing

Weak D (Du) is a weaker expression of antigen D. If a patient is antigen D negative they may be Weak D (Du) positive. Since Weak D (Du) is a weaker expression of antigen D, it will require an incubation period at a higher temperature to cause agglutination. After performing Rh testing, all negative tubes should be incubated at 37°C for 15 minutes. After incubation, antihuman globulin is added to help enhance any weak reactions, and the tubes are then centrifuged and read. If the Weak D (Du) test and patient control are negative, it is recommended that Coombs check cells be added.

Coombs check cells are reagent red blood cells coated with antibody and used to determine that the antihuman globulin (AHG) was not inactivated by substances in the patient's cell suspension. Agglutination should be noted after the addition of Coombs

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check cells. If agglutination does not occur, the AHG has been inactivated and the test is not valid. Repeat the test on a well-washed cell suspension.

It is important to run a patient control (Rh control) with the Weak D (Du) test. If the patient control (Rh control) is positive, the typing is not valid and the patient cells may need to be washed and retested, or checked for a positive direct antiglobulin test (DAT).

If the Weak D (Du) test results are negative, the patient is considered Rh D negative. If the Weak D (Du) test results are positive the patient is considered Rh D positive.

REPORTING RESULTS

There are various degrees of agglutination and all laboratories should grade the agglutination from 1+ to 4+. There are charts available that will help establish guidelines for the laboratory. Record keeping and worksheets are very important, and the results and documentation should be kept for five years for each patient.

QUALITY CONTROL

Positive and negative controls must be run and documented each day of testing. Before running controls, reagents and controls should be checked for expiration date, lot number, and turbidity. Expired or contaminated controls and reagents should not be used since they could give inaccurate or weak reactions.

Positive and negative controls may either be purchased from a manufacturer or the lab may choose to use the ABO reagents used to type the blood. The reagent A cells and B cells used for the reverse typing can be used as the negative and positive controls for Anti-A and Anti-B reagents. When using one reagent for a control of another, both reagents are simultaneously verified.

See Table 2--Control Reaction Chart for the appropriate positive and negative control reactions you should obtain for each reagent used in ABO testing.

Table 2
Control Reaction Chart

	A cells	B cells
Anti-A	Positive control	Negative control
Anti-B	Negative control	Positive control

Controls for Anti-D can be purchased from the manufacturer or verified known patients can be used. When performing Weak D (Du) testing, always confirm that the AHG is not inactivated by checking for agglutination with antibody-coated Coombs check cells.

PROFICIENCY TESTING

Laboratories that perform ABO/Rh testing should be enrolled with a proficiency testing (PT) program. If further information is needed, please refer to COLA LabFacts 8 *Proficiency Testing* or call the COLA Information Resource Center at (800) 981-9883.

SUMMARY

- When running controls for ABO testing, four tubes should be run: Anti-A with A cells, Anti-B with B cells, Anti-A with B cells, and Anti-B with A cells.
- For Rh testing use two control tubes: Anti-D with a positive control and Anti-D with a negative control. In addition, each patient should have their own control: patient cells with a D-control (Rh control), if indicated by the manufacturer.
- When performing Weak D (Du) testing, always run a patient Rh control and always confirm the AHG activity by adding Coombs check cells.

REFERENCES

1. American Association of Blood Banks. *Technical Manual*, 12th ed. Bethesda, MD. American Association of Blood Banks. 1996.
2. Harmening, D.M. Ed., *Modern Blood Banking and Transfusion Practices*, 3rd ed. Philadelphia, PA. F.A. Davis Company. 1994.