ABO BLOOD GROUPING  
(Tube Method)

I. Principle: The ABO system is the most important blood group antigen system. Individuals who lack the A and/or B antigens on their red blood cells generally have naturally occurring antibodies in their plasma that are directed against the missing antigens. For this reason, ABO grouping should include both forward (cell) and reverse (plasma or serum) procedures. Proper determination of the ABO group is necessary when selecting red blood cell-containing products for transfusion in order to prevent immediate hemolytic transfusion reactions.

II. Purpose: This test is performed to determine the ABO group of a blood sample.

1. Forward grouping uses known anti-A and anti-B reagents to demonstrate the presence or absence of the A and B antigens on the patient’s red blood cells (RBCs).

2. Reverse grouping uses known A_1 and B RBC reagents to demonstrate the corresponding presence or absence of anti-A and anti-B in the patient’s serum or plasma. (A_2 RBCs may be included, optionally)

III. Specimen

The specimen of choice is an EDTA sample that has been centrifuged to separate the plasma from the RBCs. Other acceptable anticoagulants include ACD, CPD, CP2D, CPDA-1, and heparin. A serum sample is acceptable provided there is no gel separator in the tube. Specimens should be tested as soon as possible, or stored at 1-10°C to limit deterioration of weak antibodies or false reactions due to contamination of the specimen.

IV. Equipment and reagents:

1. Anti-A and Anti-B anti-sera
2. A_1 and B red blood cells
3. 0.9% Saline
4. 12 x 75 mm test tubes
5. Dispo pipettes
6. Serofuge
7. Agglutination lamp/mirror

V. Controls:

1. Anti-A and Anti-B are tested daily against RBCs known to possess A antigen and
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B antigen respectively.

2. A1 RBC reagent is tested against anti-A, and B RBC reagent is tested against anti-B daily.

3. If an individual’s RBCs test positive with both anti-A and anti-B, as well as with anti-D from the Rh system, the cells should be tested against 6% Bovine Serum Albumin (BSA) to rule out polyagglutination and spontaneous aggregation as a cause of the reactivity. A positive test with 6% BSA invalidates the ABO test results.

VI. Procedure:  
Forward Grouping

1. Prepare a 2-5% suspension of washed RBCs from the individual to be tested, using 0.9% saline as the diluent.

2. Label 2 test tubes with specimen identifying information (patient’s name or initials or blood donor identification number [DIN]). Label one tube “A” and one tube “B”.

3. Place 1 drop of anti-A into the “A” tube and 1 drop of anti-B into the “B” tube.

4. Add 1 drop of the RBC suspension to each tube.

5. Gently shake each tube to mix the contents, then centrifuge tubes for 15-20 seconds on high speed (3500 rpm). The RBCs will form a button or pellet at the bottom of each test tube.

6. Gently resuspend the RBC button and examine for macroscopic agglutination.

7. Immediately grade the strength of the reactions and record the results on the appropriate worksheet for this test.

Reverse Grouping

1. Label 2 test tubes with specimen identifying information (patient’s name or initials or blood donor’s number). Label one tube “a” and one tube “b”.

2. Place 2 drops of the specimen’s serum or plasma into each tube.

3. Add one drop of A1 RBC reagent to the “a” tube and one drop of B RBC reagent
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to the “b” tube.

4. Gently shake each tube to mix the contents, and then centrifuge tubes for 15-20 seconds on high speed (3500 rpm).

5. Examine for hemolysis, then gently resuspend the RBC button, and read for agglutination.

6. Immediately grade the strength of the reactions and record the results on the appropriate worksheet for this test.

VII. Reporting Results

<table>
<thead>
<tr>
<th>Forward Grouping Unknown Cells Tested Against</th>
<th>Reverse Grouping Unknown Serum Tested Against</th>
<th>ABO Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>A Cells</td>
</tr>
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VIII. Additional Notes:

1. Individuals under 12 months of age may not produce a sufficient quantity of antibody to determine a reverse grouping.

2. Infants who are negative with anti-A and anti-B should also be tested with anti-A,B. Anti-A,B may be more sensitive to small quantities of antigen on the RBCs. The ABO group of a neonate (under 4 months of age) is assigned based on forward (cell) grouping results only, as ABO antibodies are usually not detectable in this age group.

3. Any discrepancy between forward and reverse grouping must be resolved before results are reported.

IX. References:
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Manufacturer’s package insert