1. **Principle**

To determine the ABO group in human blood.

ABO blood groups are determined by the presence or absence of A and B antigens on the red cells and by the presence or absence of anti-A and anti-B in the plasma.

In adults, there is a reciprocal relationship between antigen on red cells and antibody in the plasma:

|  |  |  |
| --- | --- | --- |
| ABO Group | Antigens present on red cells | Antibodies present in plasma |
| O | Neither A nor B | Both anti-A and anti-B |
| A | A only | Anti- B only |
| B | B only | Anti-A only |
| AB | Both A and B | Neither anti-A nor anti-B |

A and B antigens are detected on red cells by direct agglutination tests with commercial anti-A and anti-B blood typing reagents (forward grouping); anti-A and anti-B in plasma are detected using direct agglutination tests with known A1 and B cells (reverse grouping).

1. **Scope and Related Policies**

*Note: ABO grouping, Rh typing and antibody screen together make up a group and screen procedure.*

* 1. The ABO group shall be determined by testing the patient’s red cells with anti-A and anti-B reagents.9.1
  2. The patient’s plasma shall be tested with A1 and B reagent red cells. This test should be omitted in neonates.9.1
  3. The result of the red cell and plasma tests should agree. Any discrepancy should be investigated and resolved with appropriate documentation before issuing red cells.9.1 See NRT.003 – ABO Group Problem Solving.
  4. Previous transfusion records shall be reviewed. Previous results must be compared with current results.9.1
  5. All reagents shall be used and controlled according to the supplier’s recommendations and procedures.9.1
  6. If a discrepancy is detected and transfusion is necessary before resolution, only group O red cells shall be issued.9.2
  7. For neonatal patients (up to four months of age):
     1. A venous or capillary blood specimen should be used for all pre-transfusion testing. Cord blood must not be used for pre-transfusion testing.9.1
        1. For investigation of Hemolytic Disease of the Fetus and Newborn (HDFN), a cord blood or a venous specimen may be used to determine ABO grouping.
     2. The initial pre-transfusion blood specimen shall be tested for ABO and Rh antigens and for clinically significant antibodies.9.1 If a neonatal sample is not available, a maternal sample may be used for crossmatching and/or antibody screening.
     3. Repeat ABO and Rh typing may be omitted for the remainder of the neonatal period (up to four months) during any one hospital admission, provided that transfused red cells are group O.9.1
     4. When indicated, e.g., positive DAT or non-group O units to be selected for transfusion, IgG Anti-A and Anti-B screening of the patient’s serum / plasma should be done.
     5. If anti-A or anti-B is detected, red blood cells lacking the corresponding ABO antigen shall be transfused.
  8. Unit Testing Donor Blood: The Transfusion Service must confirm the ABO type of red cells collected and prepared by the blood supplier if a serological crossmatch will not be performed prior to transfusion.9.1For group O donor units it is only necessary to test the cells with anti-A,B.

1. **Specimens**

EDTA anticoagulated whole blood

1. **Materials**

**Equipment:** Serologic centrifuge

Block for test tubes

**Supplies:** Test tubes - 10 x 75 mm

Serologic pipettes

**Reagents:** Anti-A and anti-B commercial antisera

A1 reagent red cells

B reagent red cells

### Normal Saline

1. **Quality Control**
   1. See QCA.001 – Quality Control of Reagent Red Cells and Antisera.
   2. Reactions weaker than grade 2 in the forward ABO grouping or reactions weaker than grade 2 in the reverse ABO grouping, or mixed field reactions must be investigated.9.4

5.3 Microscopic readings should only be done if mixed field agglutination is suspected. See NRT.003 – ABO Group Problem Solving.

* 1. Plasma reacting weak or grade 1 positive with the A1 cells and/or B cells is not reliable in detecting ABO incompatibility. If crossmatching donor units by immediate spin or computer crossmatch, confirm the donor unit ABO using commercial antisera. See RT.009 – Computer Crossmatch and RT.010 – Immediate Spin Crossmatch.

1. **Procedure**

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| --- | --- |
| **STEP** | **ACTION** |
| 1. Perform a patient history check | 1. See PA.003 – Patient History Check. |
| 1. Check the suitability of the specimen(s) | 1. See PA.002 – Determining Specimen Suitability steps 6.1 – 6.4. to ensure that the specimen information matches the request form. |
| 1. Centrifuge specimen | 1. Centrifuge specimen(s) for 5 minutes at 3500 rpm or equivalent 2. Check the patient’s specimen(s) for abnormal appearance. See PA.002 – Determining Specimen Suitability step 6.5 3. Compare the patient name and identification number on all specimens with the corresponding information on the request form or computer screen. |
| 1. Label test tubes | 1. Label tubes as per established facility procedure. Example PA.004 – Labeling of Test Tubes and Block Set Up for Compatibility Testing. |
| 1. Add appropriate reagents to labeled tubes | 1. 1 drop of anti-A to the tube labeled – A. 2. 1 drop of anti-B to the tube labeled – B. 3. Add 2 drops of the patient’s plasma to the  tubes labeled A1C and BC (omit for neonate). 4. Mix the vials containing A1 and B reagent red cells and add: 5. 1 drop of A1 cells to the tube labeled A1C 6. 1 drop of B cells to the tube labeled BC   *Note: Do not pre-pipette reagent into tubes, this should be done immediately before use.* |
| 1. Prepare 3% patient red cell suspension | * + 1. See RT.003 – Preparation of 3% Patient Red Cell Suspension.   *It is not necessary to pre-wash the red cells, however, if a discrepancy is found the cells should be washed and the tests repeated* |
| 1. Add patient 3% red cell suspension to labeled tubes | 1. Add 1 drop of the patient 3% red cell suspension to the tubes labeled anti-A and anti-B. 2. Mix all tubes. 3. Centrifuge tubes at 3400 rpm for 10-15 seconds in a serological centrifuge. See Procedural Notes 8.1 4. Remove the tubes from the centrifuge in the same order in which they were placed. 5. If more than one patient is being tested, remove only the tubes for one patient. Read and record results for one patient at a time. 6. Check the name on each tube and compare with the name on the request form (or computer screen). |
| 1. Read and Interpret results | * + 1. Grade and record results See RT.001 – Reading and Recording Hemagglutination Reactions.     2. Interpret ABO grouping. See 7.0 – Reporting  |  |  | | --- | --- | | ***If*** | ***Then*** | | Patient cells and plasma react as described in the table (see Reporting 7.1) | Record the ABO interpretation on the appropriate form or in the computer as per established procedure | | Compare the ABO group obtained with the previous (historical) ABO interpretation, if available. If there is a discrepancy see NRT.003 – ABO Group Problem Solving | | Patient cells and plasma do not react as described in 7.0 – Reporting, | A discrepancy exists and must be resolved before reporting the ABO grouping. See NRT.003 – ABO Group Problem Solving. If crossmatched blood products are required STAT, before the discrepancy is resolved, select only Group O, Rh compatible, RBC units until the problem is resolved. |   6.8.3 Reactions weaker than grade 2 in the forward ABO   or reactions weaker than grade 2 in the reverse   ABO grouping must be investigated. Mixed field   reactions in the forward ABO grouping must be   investigated. In this case, do not report the ABO   group until the discrepancy is resolved. See   NRT.003 – ABO Group Problem Solving |
| 1. Perform Clerical Check | 1. For each ABO grouping, check that:  * The patient name and identification number are identical on all specimens and on the request form * The patient name is the same on all test tubes and on the request form * The test results have been interpreted correctly |
| 1. Complete paperwork | 1. Initial or sign and record the completion time and date on the appropriate form or in the computer 2. Verification of results must be recorded |

1. **Reporting**
   1. Hemagglutination tests should react as follows:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Patient cells vs | | Plasma vs | | Interpretation |
| ANTI-A | ANTI-B | A1 CELLS | B CELLS | GROUP |
| Neg | Neg | = or > 2 | = or > 2 | O |
| = or > 2 | Neg | Neg | = or > 2 | A |
| Neg | = or > 2 | = or > 2 | Neg | B |
| = or > 2 | = or > 2 | Neg | Neg | AB |

“= or >”: equal to or greater than

* + 1. Antisera (-A and/or –B) and patient cells should react grade 2 or stronger (forward group).9.4
    2. A1 and B reagent red cells should react grade 2 or stronger with patient plasma (reverse group).9.4
    3. Hemolysis is an acceptable reaction in reverse group. Hemolysis must be interpreted as a positive reaction.
  1. For specimens showing positive reactions with Anti-A and Anti-B e.g. AB a concurrent control must be performed on the patient’s cells

1. **Procedural Notes**
   1. Centrifuge the tubes:
      1. If only one patient specimen is being tested, place the tubes

labeled –A and –B in one side of the serological centrifuge and the tubes A1C and BC on the other side.

* + 1. If more than one patient is being tested, keep each patient’s set of tubes in the following order: -A, -B, A1C, BC. Place each set of tubes opposite to each other to balance the serologic centrifuge.
  1. Haematopoietic cell (HPC) (collected by apheresis, cord or bone marrow) transplant : In cases of ABO discrepancies where the patient is a known recipient of an ABO incompatible HSCT, the ABO discrepancy does not have to be resolved in the traditional manner. The ABO interpretation should be resulted as “NOTE” with chartable footnote “HEMATOPOIETIC CELL (HPC) TRANSPLANT– BLOOD GROUP IN TRANSITION”. The charge technologist will monitor the ABO grouping of these patients. Once the patient’s new stem cells have engrafted (conclusive ABO testing on two occasions 6 months post transplant), the charge technologist will change the patient ABO to the new group. After engraftment the reverse grouping may never correspond with the forward grouping, again the discrepancy does not have to be resolved. Once the charge technologist has changed the patient’s historical ABO, the ABO interpretation should be resulted based on the forward grouping results with a footnote indicating the source of the transplant

e.g. “HEMATOPOIETIC CELL (HPC) TRANSPLANT RECIPIENT”.

1. **References**
   1. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine, 5.2.5.1, 5.3.1, 5.3.2, 5.3.7, 5.9.2.
   2. Standards for Blood Banks and Transfusion Services, 28th ed. Bethesda, MD: AABB, 2012; 5.13.1, 5.16.
   3. Roback JD, ed. Technical Manual, 17th ed. Bethesda, MD: AABB, 2011: 445, 801, 876.
   4. Judd WJ. Methods in Immunohematology, 3rd ed. Bethesda, MD: American Association of Blood Banks, 2008; 2-6.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| January 31, 2014 | * Revised name of manual * Changed document number from RT 001 to RT 004 * Revised wording in section 2.8 to include “The Transfusion Service must confirm the ABO type of red cells collected and prepared by the blood supplier if a serological crossmatch will not be performed prior to transfusion.9.1” * Revised reference made in section 6.8 from RT 014 to RT 003 * Revised reference made in section 6.14 from PA 006 to RT 001 * Added section 7.2 with reference to specimens showing a positive reaction with Anti-A and Anti-B * Revised wording of section 8.2 to include “indicating the source of the transplant” * Updated list of references to include the most recent editions/versions |