1. **Principle**

To determine the Rh (D) type in human blood.

Rh (D) type is determined by the presence or absence of the D antigen on red cells. D antigen is detected on cells using a direct agglutination test with anti-D reagent.

1. **Scope and Related Policies**

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

* 1. The Rh type shall be determined by testing the patient’s red cells with anti-D reagent. 9.1
  2. If an Rh typing problem is detected and transfusion is necessary before resolution, for pre-menopausal females and children Rh negative blood products must be issued until the problem is resolved. Other patients, in the absence of a known anti-D, may receive Rh positive blood in emergency situations when there is a shortage of Rh negative blood.
  3. When testing potential blood product recipients, the test for weak D is unnecessary except as stated in 2.7.1.9.1
  4. Obstetrical patients that type as Rh positive or weak D positive should be designated as Rh positive. Patients whose red cells type as Rh negative should be designated as Rh negative.9.1
  5. Previous transfusion records must be reviewed. Previous results must be compared with current results.9.1
  6. A control system, appropriate to the anti-D reagent in use, shall be included.9.1 If this control is positive, the Rh typing must be repeated with an appropriate anti-D reagent and control
     1. Although some manufacturers do not recommend an Rh control, all manufacturers list as a test limitation the possibility that false positive reactions may occur if the test red cells are strongly agglutinated prior to the addition of the reagent. This is also true with bacterial contamination of the specimen. Therefore an Rh control should be tested concurrently with anti-D antisera. See Quality Control 5.3.
  7. For neonatal patients:
     1. Testing for weak D shall be performed on infants who type as Rh negative if their mother is Rh negative and has no evidence of Rh alloimmunization.9.1
     2. 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 other purposes (e.g., investigation of Hemolytic Disease of the Fetus and Newborn or to assess the need for injection of Rh Immune Globulin to the mother), a cord blood or a venous specimen may be used to determine Rh typing.
     3. The initial pre-transfusion blood specimen shall be tested for ABO and Rh antigens and for clinically significant antibodies.9.1 For neonatal patients these tests may be performed on the maternal sample for crossmatching and/or antibody screening.
     4. Repeat ABO and Rh typing may be omitted for the remainder of the neonatal period (up to four months) during any one hospital admission.9.1
  8. For apparent Rh negative obstetrical patients testing for weak D may be performed as per facility policy.

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-D monoclonal IgM blended with polyclonal anti-D polyclonal anti-D

Control for Rh typing (appropriate for reagent in use   
 according to manufacturer’s directions)9.2

# Normal Saline

1. **Quality Control**
   1. See QCA.001 – Quality Control of Reagent Red Cells and Antisera.
   2. If blood components are required STAT, Rh negative red blood cells (RBC) should be selected for pre-menopausal females and children. Other patients, in the absence of a known anti-D, may receive Rh positive blood in emergency situations when there is a shortage of Rh negative blood.
   3. False positive results in the Rh control due to cold autoagglutinins or a protein imbalance may occur if testing with unwashed red cells.
      1. Absence of this spontaneous agglutination can usually be demonstrated by observing negative reactions in the forward ABO grouping (reactions with anti-A and/or anti-B).
      2. For specimens that show agglutination in all tubes (i.e., give the reactions of group AB, D positive), a concurrent control must be performed on the patient’s cells. This is not required for donor unit confirmation.
      3. If a commercial control for low protein reagent (monoclonal/polyclonal blended) is not available, autologous plasma or a 6% bovine albumin control may be used.
2. **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 2. Centrifuge specimen for 5 minutes at 3500 rpm or equivalent. 3. Check the specimens for abnormal appearance. See PA.002 – Determining specimen Suitability steps 6.5 – 6.6 |
| 1. Label tubes | 1. Label tubes as per established facility procedure. Example PA.004 – Labeling of Test Tubes and Block Set Up for Compatibility Testing. See Quality Control – 5.2 and Procedural Notes 8.1 |
| 1. Add reagents | 1. Add 1 drop of anti-D to the tube labeled D. 2. Add 1 drop of Rh control reagent (if applicable) to the tube labeled DC |
| 1. Prepare a 3% patient red cell suspension | 1. Compare the patient name and identification number on each tube with the corresponding information on the request form (or computer screen) to ensure they are the same 2. See RT.003- Preparation of a 3% Red Cell Suspension.   ***Note: 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 1 drop of the 3% red cell suspension to the corresponding tubes labeled D and DC (if applicable). 2. Mix tubes 3. Centrifuge tubes at 3400 rpm for 10-15 seconds in a serologic centrifuge. See Procedural Notes 8.2. 4. Remove the tubes from the serologic centrifuge in the same order in which they were placed. If more than one patient is being tested at a time, remove only the tubes for one patient. Read and record results for one patient at a time |
| 1. Read and grade results | 1. Read results as per established procedure. See RT.001 – Reading and Recording Hemagglutination Reactions. 2. Grade and record results |
| 1. Incubate negative tubes | 1. Incubate negative results and repeat 6.5.5 to 6.6.2 if indicated in the manufacturer’s insert |
| 1. Interpret results | 1. Interpret the Rh type. See 7.0 – Reporting. 2. Compare the Rh group obtained with previous (historical) Rh typing interpretation, if available. If there is a discrepancy see NRT.004 – Rh Typing Problem Solving  |  |  | | --- | --- | | ***If*** | ***Then*** | | patient cells react as described in  7.0 - Reporting | Record the Rh typing on the request form or in the computer as per established facility procedure. | | patient cells do not react as described in 7.0 – Reporting | A discrepancy exists and must be resolved before reporting the Rh type. See NRT.004 –Rh Typing Problem Solving. If crossmatched blood products are required STAT, before the discrepancy is resolved, select only Rh negative RBC units until the problem is resolved.  See Scope and Related Policies 2.2 and Quality Control 5.2. | |
| 1. Perform a clerical check | 1. Check when the procedure is complete 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 or in the computer * Verify that the test results have been interpreted correctly |
| 1. Complete paperwork | 1. Initial or sign and record the completion time and date on the appropriate request form or in the computer. 2. Verification of results must be recorded. See 7.0 Reporting. |

1. **Reporting**
   1. For valid Rh type, if a control is required, the control tube result must be negative. Rh type should be reported as follows: 9.3, 9.4
      1. Agglutination (grade 2 or stronger) with anti-D indicates the presence of the D antigen. Report as Rh positive.
      2. No agglutination with anti-D indicates the absence of the D antigen. Report as Rh negative.
   2. If a control is required and is positive or if the reaction with anti-D is weak, mixed field or grade 1, do not report the Rh type.
      1. Additional work is required to investigate the positive control or the weak reaction with anti-D. See NRT.004 – Rh Typing Problem Solving.
2. **Procedural Notes**
   1. If using a high protein antisera (slide and modified tube anti-D), to facilitate the detection of false positive reactions with the anti-D reagent, a control should be set up when performing an Rh typing.9.2
   2. Centrifuge the tubes.
      1. If only one patient specimen is being tested, balance the one tube in the centrifuge with using a blank extra tube for balance.
      2. If more than one patient is being tested, place each tube opposite to each other to balance the serologic centrifuge.
3. **References**
   1. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine, 5.3.1.4, 5.3.3.1, 5.3.3.3, 5.3.3.2, 5.9.2.1-5.9.2.4.
   2. Transfusion Medicine Review – Rh Typing. QMPLS ver 1 2013-10-10: 1-7.
   3. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: AABB, 2011: 445-448-, 885-886.
   4. Judd WJ. Methods in Immunohematology, 3rd ed. Bethesda, MD: American Association of Blood Banks, 2008: 2-6.
   5. Standards for Blood Banks and Transfusion Service, 28th ed. Bethesda, MD AABB. 2012: 5.13.2.
4. **Revision History**

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| **Revision Date** | **Summary of Revisions** |
| January 31, 2014 | * Revised name of manual * Section 4.0 – added to control for Rh Typing ‘appropriate for reagent in use according to manufacturer’s directions’ * Section 6.0 – 6.8 RT.014 changed to RT.003; 6.14 PA.006 changed to RT.001. * Revised the list of references and updated to include most recent version/ edition. Added QMPLS reference. |