1. **Principle**

To resolve Rh typing problems

1. **Scope and Related Policies**
	1. Rh typing should be investigated when:
* The Rh control test is positive
* Results are weak or less than grade 2 positive with anti-D reagent. Microscopic readings should only be done if mixed field agglutination is suspected.
* Rh typing discrepancies are found between current and previous results.
	1. Current testing results shall be compared with previous records to identify any discrepancies. All discrepancies shall be resolved prior to reporting.9.1
	2. All reagents shall be used and controlled according to the manufacturers written instructions.9.1
	3. If an Rh typing problem is detected and transfusion is necessary before resolution, for females of childbearing age and children, Rh negative blood products must be issued until the problem is resolved. When there is a shortage of Rh negative blood other patients, in the absence of a known anti-D, may receive Rh positive blood in emergency situations.
1. **Specimens**

EDTA anticoagulated whole blood

1. **Materials**

**Equipment:** Serological centrifuge

 Block for test tubes

 Microscope

**Supplies:** Test tubes – 10 x 75 mm

 Serological pipettes

**Reagents:** Control for anti-D, if applicable

 Anti-D reagent

 Normal saline

1. **Quality Control**
	1. See QCA.001 – Quality Control of Reagent Red Cells and Antisera.
	2. An Rh control may be required for Rh tests (refer to anti-D manufacturer’s insert).
		1. A negative reaction with anti-A or anti-B usually rules out spontaneous agglutination
		2. 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.
		3. False positive results in the Rh control may occur if the plasma contains autoagglutinins or a protein imbalance causing rouleaux is present and the red cells are tested unwashed.
		4. A DAT using IgG reagent should be tested as a control for selected samples (e.g., cord, neonatal and samples demonstrating autoagglutination) in order to rule out false negative Rh typing due to blocked antigens.
2. **Procedure**

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| 1. Preliminary Checks
 | 1. Recheck suitability of specimen(s). See PA.002 – Determining Specimen Suitability. If the specimen is unsuitable, collect another specimen and repeat the Rh typing
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| 1. Obtain and record the patient’s diagnosis and transfusion/obstetrical history.
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| 1. Check the label on the vial(s) of reagent to ensure that the correct reagent was used.
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| 1. Prepare a new 3% patient red cell suspension.
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| 1. Repeat the Rh typing on the new 3% patient cell suspension.

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| ***If*** | ***then*** |
| problem is solved | see Procedural Notes 8.1 and proceed to step 6.4 |
| problem remains and transfusion is required STAT | select Rh negative donor units. See Procedural Notes 8.8 |

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| 1. Identify the Rh problem and proceed to the appropriate step(s)
 | 1. The reaction with anti-D is demonstrating **mixed field** and the control is negative:

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| ***If*** | ***then*** |
| patient has been transfused in the last three months with blood of a different Rh group | * Read the tubes microscopically and look for mixed field agglutination and record results
* Record explanation for the discrepancy (e.g., Patient transfused with\_\_\_\_ units of Rh positive RBC on \_\_\_\_).
* A large fetomaternal bleed can also cause mixed field agglutination
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| patient has not been transfused in the last three months | perform a weak D typing test. See RT.006-Weak D Typing. |

* See Procedural Notes 8.2 for other possible causes of mixed field agglutination
* If Rh typing is resolved, proceed to step 6.4
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| 1. The reaction with anti-D is demonstrating **mixed field** and the control is positive:
2. Wash the cells 4 times with normal saline (if cold agglutination is suspected, use 37° C normal saline to wash the cells). See RT. 002 – Cell Washing Automated and Manual.
3. Repeat the Rh typing on a freshly prepared 3% washed red cell suspension
4. If the control is negative when using a 3% washed red cell suspension, report the Rh typing. See Procedural Notes 8.3 for possible causes. Proceed to step 6.4.

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| ***If*** | ***Then*** |
| control is still positive using washed red cells | perform a Direct Antiglobulin Test (DAT). See RT.007 – Direct Antiglobulin Test. |
| the DAT is positive | repeat the Rh typing using monoclonal anti-D and a 6% BSA control. |
| the control is negative | report the Rh type |
| the control is positive | perform chloroquine treatment to remove the antibody coating the cells. Follow the manufacturer’s directions for chloroquine treatment of cells. An EGA kit may be used as an alternative or refer the specimen out for investigation.Retest the Rh using chloroquine/EGA treated cells. (See SP.011 Dissociation of IgG by Chloroquine Diphosphate.) |
| the discrepancy is resolved | report the Rh type with a note that the test was done using chloroquine/EGA treated cells and proceed to step 6.3 |
| the discrepancy is not resolved | refer the specimen to a reference lab for additional testing |

1. If the results are discrepant (previous Rh typing does not agree with the current test result):

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| ***If*** | ***Then*** |
| the patient was transfused in the last three months with a different Rh type | follow steps 6.2.1 |
| the patient has not been transfused in the last three months and discrepancy cannot be resolved by testing for weak D antigen | recollect a specimen and repeat the test. |
| the result on the new specimen is the same as the result on the discrepant specimen | consider the following possibilities:* A collection error might have occurred with the historical sample
* A technical or clerical error on the previous specimen. If possible, review the historical test results to ensure that an interpretation error did not occur. Proceed to 7.0 – Reporting
* Patient is using another person’s identification
* Loss of antigen due to disease. Check the diagnosis. See Procedural Notes 8.6
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| the result on the new specimen is different from the result on the discrepant specimen and matches the historical record | consider the possibility an error in collection of the previous sample occurred:* Check into the possibility that other patients are involved (e.g., another patient specimen collected close to the same time by the same phlebotomist)
* Complete an incident report and submit to a supervisor
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| 1. Report Process
 | 1. Report the process and the conclusion of the investigation on the request form or computer
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| 1. Perform clerical check
 | 1. Ensure that the specimen label information for each specimen tested coincides with the information on the corresponding test tubes and request form
 |
| 1. Initial
 | 1. Sign or initial and record the completion time and date on the request form or complete the computer record
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1. **Reporting**
	1. Record
* All procedures that were used to resolve the Rh typing problem (e.g., washing the cell suspension, use of different anti-D, etc.)
* All observed test reactions shall be documented.9.1
	1. When a patient previously typed as Rh negative is found to be Rh positive after investigation, the patient Rh type should be reported as (for example): This sample has been confirmed to be Rh positive.
	2. When a patient previously typed as Rh positive is found to be Rh negative after investigation, the patient Rh type should be reported as (for example): This sample has been confirmed to be Rh negative.
1. **Procedural Notes**
	1. If the discrepancy has been resolved, one of the following could have caused the discrepancy:
* Procedural errors
* Antiserum not added, or wrong one used
* Cell suspension too strong
* Centrifugation insufficient or excessive
* Tube shaken too vigorously and small agglutinates were dispersed
* Failure to resuspend entire cell button
* Reading microscopically when antisera instructions indicate macroscopic reading.
	1. Possible causes for mixed field agglutination:
* Recent transfusion with different Rh donor unit
* Recent allogeneic bone marrow or hematopoietic stem cell transplant
* Contaminated specimen
* Unusual Rh phenotype that may or may not be associated with production of Rh alloantibodies
* Large fetomaternal bleed
* Genetic anomalies such as dispermy and chimerism.
	1. Positive control may be due to:
* Rouleaux
* Strong Autoagglutinins
* Positive Direct Antiglobulin Test (DAT).
	1. If the anti-D and Rh control are high-protein reagents, the Rh typing should be repeated using a low-protein reagent such as monoclonal anti-D and control. If the low-protein control is still positive, the antibody coating the cells should be removed before repeating the Rh typing.
	2. False positive reactions with anti-D (control negative) may be caused by the presence of antibodies to antigens of low frequency in antisera of human origin. Repeating the Rh typing with monoclonal reagents should resolve the problem. Monoclonal reagents do not have contaminating antibodies.
	3. False negative reactions with anti-D may occur:
* When the cells are coated with an antibody specific for the antigen being tested. Example: when the D antigen sites on fetal cells are coated with the maternal anti-D, the neonate’s red cells may fail to react with the anti-D and therefore type as Rh negative.
* In disease states that result in loss of antigen. Patients who were known to be D positive have been studied during their illness and were found to be D negative by “weak D typing” test. RHD genotyping is useful to discriminate partial D, or specific weak D or to resolve serological D typing.9.2
	1. Weak reactions with anti-D may result from RHD gene variations that result in reduced expression of the D antigen. Monoclonal anti-D reagents are designed to react with DIV and DV variants. DVI variants will be detected using IAT tests. Check the manufacturer’s insert for the reagent in use. An individual with a partial D should be considered Rh positive as a blood donor but Rh negative as a transfusion patient.9.2
	2. If an Rh typing problem is detected and transfusion is necessary before resolution, for females of childbearing age and children, Rh negative blood products must be issued until the problem is resolved. When there is a shortage of Rh negative blood other patients, in the absence of a known anti-D, may receive Rh positive blood in emergency situations.
1. **References**
	1. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine; 5.3.1.1, 5.3.1.3, 5.3.1.4, 5.3.3
	2. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011; 406-408
	3. QMP-LS EQA Transfusion Medicine Review- Rh Typing 2013-10-10
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised name of manual
* Revised wording in section 2.2 to include reference to resource 9.1 “Current testing results shall be compared with previous records to identify discrepancies. All discrepancies shall be resolved prior to reporting.9.1”
* Revised wording in section 2.3 to include “according to the manufacturers written instructions”
* Specified females “of childbearing age” in sections 2.4 and 8.8
* Revised wording in section 5.2.4 to specify a DAT “using IgG reagent”
* Added section 6.1.2 “Obtain and record the patient’s diagnosis and transfusion/obstetrical history”
* Section 6.2.1 changed RT.003 to RT.006; Section 6.2.2 changed RT. 004 to RT. 007; Section 6.2.2.1 changed PA.005 to RT. 002; Section 6.2.3 changed Procedural Notes 8.2 to Procedural Notes 8.6
* Revised wording of section 7.1 to include “all observed test reactions shall be documented.9.1”
* Added reference to 9.2 in section 8.6 “ RHD genotyping is useful to discriminate partial D, or specific weak D or to resolve serological D typing.9.1”
* Updated list of references to include most recent editions/versions.
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