1.0 Principle

To detect Rh positive fetal cells in a blood specimen from an Rh negative woman.

The rosette test is a screening test performed to detect Rh positive cells in an Rh negative population. Red blood cells from an Rh negative woman are incubated with anti-D. During incubation, anti-D will sensitize Rh positive fetal cells if present in the test suspension. Excess anti-D is removed by washing. Enzyme-treated cDE indicator cells are added and will bind to any anti-D-sensitized Rh positive cells present in the original specimen to form rosettes. Rosettes can be seen by microscopic examination.

2.0 Scope and Related Policies

2.1. All reagents shall be used and controlled according to the supplier’s recommendations and procedures. 9.1 (F1.1), 9.2 (8.1.2)

2.2. A test shall be performed to determine the amount of a fetal-maternal bleed in non-alloimmunized Rh negative women who have delivered an Rh positive infant. 9.1 (H5.8), 9.2 (11.9.6) If a fetal bleed is detected, an appropriate dose of RhIG shall be given according to the supplier’s recommendations. 9.1 (H5.8), 9.2 (11.9.6)

2.2.1. A post-partum maternal blood sample shall be tested to detect a significant fetomaternal hemorrhage requiring more than a single dose of Rh Immune Globulin for effective prophylaxis. 9.2 (11.9.6)

2.2.2. When the rosette test is positive, a quantitative test (i.e., Kleihauer-Betke test) is required to determine the volume of the fetomaternal hemorrhage as a means of estimating the dose of RhIG needed to prevent Rh immunization. 9.2 (11.9.6)

2.2.3. A rosette test is done only on a post-partum specimen from an Rh negative woman who has delivered an Rh positive neonate. 9.2 (11.9.6)

3.0 Specimens

Maternal EDTA collected post-partum before administration of RhIG (preferably 1 hour post-partum).
4.0 Materials

Commercial kit that includes anti-D, a positive control, a negative control and indicator cells (cDE enzyme treated)
Buffered saline
Test tubes
Pipettes
Test tube racks
Serologic centrifuge
Cell washer
Microscope

5.0 Quality Control/Management

5.1. The positive and negative controls shall be set up with each batch to confirm that the proper reagents have been added and that the red cell and anti-D mixture has been washed adequately.

6.0 Procedure

6.1. Check the suitability of the specimen(s). See PA.002 - Determining Specimen Suitability.

6.2. Confirm that the specimen has been collected **post-partum** and before administration of RhIG.

6.2.1. If the recipient has received RhIG after delivery and before the collection of a post-partum specimen, perform an antibody screen and a Kleihauer-Betke test on the post-partum specimen.

6.2.1.1. If the antibody screen reveals an anti-D and no fetal cells are detected in the Kleihauer-Betke test, additional RhIG is **not** required.

6.2.1.2. If the antibody screen does not reveal an anti-D or if the Kleihauer-Betke test reveals a large fetal bleed, additional RhIG may be required.

6.2.1.3. Proceed to 6.20.

6.3. Mix maternal specimen(s) well.

6.4. Prepare a **3-5%** suspension.
6.4.1. Label a test tube with the FULL last name; transcribe the last name **from the specimen tube**, not from the request form. A pre-printed label may be used (ensure the information coincides exactly with the specimen label).

6.4.2. Dispense 2 drops of whole blood into the labelled tube.

6.4.3. Add 0.5 to 1.0 mL of saline and mix to resuspend to 3%. Compare with one of the control cell suspension. The concentration of the recipient cell suspension should be comparable with the concentration of the positive control cell suspension.

6.5. Label tubes:

- one tube(s) with the recipient name or accession number and test name (e.g., ROS).
- 1 tube: “Pos con”
- 1 tube: “Neg con”.

6.6. Dispense 1 drop of anti-D reagent into each tube.

6.7. Pipette 1 drop of the appropriate 3–5% cell suspension to the corresponding labelled tube and mix well.

6.8. Incubate the tubes at 37°C for 15 minutes.

6.9. Check and record the temperature of the water bath or heating block on form QCAI.006F.

6.10. After incubation, remove the tubes from the water bath or heating block and wash 4 times.

   Attach or tape a note (e.g., Rosette) on the cell washer to indicate that rosette tests are being washed. This should prevent the addition of the wrong reagent (i.e., antihuman globulin reagent) upon opening of the cell washer.

6.11. Mix the vial of indicator cells well.

6.12. Add 1 drop indicator cells to each tube and mix.
6.13. Centrifuge the tubes at 3,400 rpm for 15 seconds (or as recommended in the manufacturer’s insert).

6.14. Gently resuspend the button of cells and read microscopically. If desired, once resuspended, the cells may be poured onto a labelled slide for reading.

6.15. Examine at least 10 fields microscopically (at x100 power). Count and record the number of rosettes seen in each field.

6.16. Interpret and report the results. See 7.0 – Reporting.

6.17. If the rosette test is positive, order a Kleihauer-Betke test to determine the amount of RhIG that should be given.

6.18. When the procedure is complete, do a clerical check. Check that the:

- Name and hospital number are identical on all specimens and on the request form
- Name is the same on the test tubes and on the request form
- Test results have been interpreted correctly.

6.19. Initial or sign and record the completion time and date on the request form.

6.20. Prepare correct dosage of Rh Immune Globulin, if required. See procedural note 8.3.

### 7.0 Reporting

7.1. Test results may be interpreted and reported only when the positive and negative controls reacted correctly.

Rosettes (or agglutinated cells) shall be seen in the positive control.

No rosettes (or less than 3 per 10 fields) shall be seen in the negative control.

7.2. If the total number of rosettes seen in 10 fields is 3 or more, interpret and report as "Rosette test positive". A Kleihauer-Betke test shall be performed to determine the volume of fetal cells. See procedural notes 8.1 and 8.2.
7.3. If the total number of rosettes seen in 10 fields is less than 3, interpret and report as “Rosette test negative”.

**8.0 Procedural Notes**

8.1. The number of rosettes observed should not be used as a means to quantitate the amount of the fetomaternal hemorrhage.

8.2. If a rosette test result is strongly positive (i.e., is macroscopically positive) the maternal cells are probably "D positive".

In this case, repeat the Rh typing on the maternal specimen (preferably a pre-delivery specimen) and if negative, perform a test for weak D typing and a Kleihauer-Betke test.

8.2.1. If the test for weak D is positive and the Kleihauer-Betke test is negative, the maternal cells are probably "D positive".

Report: "Recipient is Rh positive. Rosette test not valid."

8.2.2. If the test for weak D is positive and the Kleihauer-Betke test is positive refer to a pathologist regarding the need for Rh Immune Globulin.

8.3. When the rosette test is negative, prepare a dose of Rh Immune Globulin as recommended by the Society of Obstetricians and Gynaecologists of Canada (120–300 µg within 72 hours of delivery) or according to hospital policies and procedures for administration of Rh Immune Globulin.

If the rosette test is positive, prepare dosage of Rh Immune Globulin as determined by the Kleihauer-Betke test result.

**9.0 References**


Facility endorsement if guideline is used as a Standard Operating Procedure (SOP)

Approved By: 
(Senior Management)

(Senior Management)

Facility effective date: DDMMYY
(Date of implementation)

Change Log

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<thead>
<tr>
<th>Change Description</th>
<th>Effective Date</th>
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<td>01 April 2000</td>
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<tr>
<td><strong>Revision 1</strong></td>
<td></td>
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<tr>
<td>Changed title (from Test for Fetomaternal Hemorrhage).</td>
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<tr>
<td>Rearranged scope and related policy.</td>
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<tr>
<td>Added heating block whenever waterbath is documented</td>
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<tr>
<td><strong>6.17 - 6.19:</strong> Added steps to report result, initial</td>
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<tr>
<td>worksheet and prepare Rh Immune Globulin for issue.</td>
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<tr>
<td><strong>7.2:</strong> wording changed to test or send out K-B test.</td>
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<tr>
<td>Previous 8.1: Moved to QC 5.2 and reference in 6.2.1.</td>
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<td>changed accordingly.</td>
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<tr>
<td>Added procedural note 8.3 concerning preparing Rh</td>
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<td>Immune globulin.</td>
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<td><strong>9.2:</strong> Updated reference.</td>
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<td><strong>Revision 2</strong></td>
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<td>Changed “patient” to “recipient”, “must” to “shall” and</td>
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<td>updated to CAN/CSA Z902-04 in all cases</td>
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<td>where applicable</td>
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<td><strong>5.0:</strong> Updated title</td>
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<td><strong>8.3:</strong> For negative rosette tests, changed “a 600 IU</td>
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<td>policy states otherwise, a 1500 IU dose of Rh</td>
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<td><strong>9.0:</strong> Deleted references 9.2–9.5; added new</td>
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