1. **Principle**

To remove antibody from coated red cells.

Freeze – thaw elution techniques are usually restricted to the confirmation of ABO hemolytic disease of the fetus and newborn (HDFN) because they rarely work well for detection of other antibodies.9.1

Red cells coated with antibody are thoroughly washed to remove unbound protein. The washed cells are then frozen and subsequently rapidly thawed. The hemolysate is then tested for the presence of the suspected antibody.9.2

1. **Scope and Related Policies**
   1. This elution may be performed to investigate fetal-maternal ABO incompatibility. This method is notto be used routinely for other investigations.
   2. When the Direct Antiglobulin Test (DAT) is found to be positive in a cord or newborn sample and anti-A or B is suspected an elution may be performed to confirm the presence of the antibody as per facility policy.
   3. All reagents shall be used and controlled according to the manufacturer’s written instructions. 9.3
2. **Specimen**

Cord blood clotted specimen

1. **Material**

**Equipment:** Cell Washer

Serological centrifuge

Block for test tubes

Freezer (at least minus 20° C)

Microscope

**Supplies:** Test tubes – 10 x 75 mm

Serological pipettes

Stopper

**Reagents:** Normal saline

IgG check cells

Screening cells

A1 and B cells

1. **Quality Control**
   1. The last wash is tested with the eluate to ensure that the recovered antibody present in the eluate has been released from a bound state on the original cells and not residual unbound antibody remaining as a result of inadequate wash procedure.
2. **Procedure**

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| * 1. Centrifuge the specimen for 5 minutes at 3500 rpm or equivalent.  Label three tubes with first three letters of the patient's family name. Transcribe the information from the patient specimen label(not from the request form). Patient specimen labels may be used (ensure the information coincides exactly to the specimen label). |
| * 1. Label one tube serum, one tube eluate and one tube last wash. |
| * 1. Separate the serum and add to the tube labeled serum. |
| * 1. Add 20 drops of red cells to the tube labeled eluate. Wash the red cells 6 times with normal saline. |
| * 1. Add last wash to the appropriately labeled tube and retain for further testing. |
| * 1. To 0.5 mL washed packed cells add 3 drops of normal saline. Mix well and stopper securely. |
| * 1. Rotate the tube in order to coat the inside of the tube with red cells. |
| * 1. Place the tube on its side in a freezer (-20°C to -70°C) for 10 minutes (or longer) until frozen. |
| * 1. Thaw the red cells rapidly by holding the tube under warm running tap water. |
| * 1. Centrifuge the thawed cell hemolysate at 1000 rpm for 2 minutes to clear of cellular debris and transfer the supernatant eluate to a clean test tube labeled with the patient’s family name and “eluate” |
| * 1. Label 2 sets of 5 tubes:   For the eluate: A1, B, and screening cells For the last wash: A1, B, and screening cells  To the set labeled eluate add 2 drops of the hemolysate To the set labeled last wash add 2 drops of last wash |
| * 1. To each labeled tube add 1 drop of the appropriate 3% red cell suspension. |
| * 1. Incubate all tubes at 37°C for 30 minutes. |
| * 1. Wash cells 4 times see RT.002 Cell Washing Automated and Manual. |
| * 1. Add 2 drops of anti IgG to each tube and centrifuge at 3400 rpm for 10-15 seconds. |
| * 1. Read and record the results. See RT.001- Reading and Recording Hemagglutination Reactions. |
| * 1. Interpret and Report the results. See 7.0 – Reporting. |

1. **Reporting**
   1. The last wash must be negative for the test to be valid.
   2. If an antibody is identified in the eluate, report: “Eluted antibody, anti-\_\_\_\_ (A and/or B)”.
   3. If the eluate is negative against the screening, A and B cells and/or panel cells, report: “Negative.” See Procedural Notes 8.2.
2. **Procedural Notes**
   1. If antibody is present in the last wash, i.e., washing of the cells was inadequate and failed to remove free antibody, the results of the eluate cannot be reported. The eluate should be repeated with additional washing of the cells in step 6.4
   2. If the eluate is negative confirm that the DAT was positive with anti-IgG. If the DAT is confirmed positive consider an antibody to a low frequency antigen and select low frequency positive cells to test against the eluate.

1. **References**
   1. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 479.
   2. Judd WJ, Methods in Immunhematology 3rd ed.:2008: 140-142
   3. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine, 5.3.1.1.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised manual name * Revised wording of section 6.2 to include “label one tube serum” * Revised wording of section 6.10 to include “labeled with patient’s family name and eluate.” * Section 6.14- changed PA.005 to RT.002; Section 6.16- changed PA.006 to RT.001 * Updated list of references to include latest editions/versions |