1. **Principle**

Newly formed autologous red cells (reticulocytes) have a lower specific gravity than transfused red cells and may be separated from the transfused population by simple centrifugation. The autologous red cells will concentrate at the top of a centrifuged tube. The density of red cells increase as the cells age, allowing separation of young and old red cells based on their relative densities. During high-speed centrifugation, the youngest less dense cells will remain in the top layer, while the older more dense cells will go to the bottom of the red cell sample.

In a red cell sample, the youngest red cells, the reticulocyte rich portion (RR) are usually autologous red cells. The older red cells, the reticulocyte poor portion (RP) are autologous and if present, transfused donor red cells

1. **Scope and Related Policies**
   1. Separation of autologous RBC's should be attempted when:

* phenotyping of autologous cells is necessary
* to determine whether a positive DAT is due to a delayed hemolytic transfusion reaction (DHTR) or an Autoimmune process
* when DAT positive cells cannot be reduced to negative by routine procedure and phenotyping by IDAT is required
  1. The method chosen will depend upon the volume of the sample, centrifuge equipment available and the time frame.

1. **Specimen**

EDTA anticoagulated whole blood preferably less than 24hours old.

1. **Materials**

**Equipment:** Fisher high-speed centrifuge (7000 G capacity)

**Supplies:** Fisher polystyrene micro centrifuge tubes

(#4-978-145)

Serological pipettes

**Reagents:** N/A

1. **Quality Control – N/A**
2. **Procedure**

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| * 1. Centrifuge the patient specimen for 10 minutes at 3000 rpm or equivalent. |
| * 1. Remove the plasma without disturbing or aspirating the red cells. |
| * 1. Stopper and mix red cell sample thoroughly. |
| * 1. Label conical plastic microtubes with the patient’s full name. Transcribe the information from the patient specimen label(not from the request form). The number of tubes will depend on the volume of red cells. |
| * 1. Cap tubes and centrifuge at 7000G for one hour. |
| * 1. Label two 10 x 75 mm tubes one TOP and the other BOTTOM and with the first three letters of the patient's last name. |
| * 1. Following centrifugation carefully remove the tubes taking care not to disturb the red cells. |
| * 1. Using a Pasteur pipette, skim the top layer of red cells. Do not apply suction. Allow red cells to rise in the pipette by capillary action. Rinse the pipette with saline in the tube labelled TOP. Collect top layers in a similar manner from all of the micro tubes. |
| * 1. With a clean pipette, depress rubber bulb and insert tip of the pipette to the bottom of tube. Apply gentle suction and withdraw a small volume of red cells from the bottom of the tube and rinse pipette with saline in the tube labeled ‘BOTTOM’. |
| * 1. Test the RR (TOP) and the RP (BOTTOM) and the unseparated red cells in parallel for DAT and red cell phenotypes. See RT.007- Direct Antiglobulin Test and NRT.009 Antigen Typing – Direct and Indirect Agglutination. |
| * 1. Careful observation of the RR for mixed field agglutination is necessary to ensure that cell separation was effective in isolating autologous red cells. |

1. **Reporting – N/A**
2. **Procedural Notes**
   1. The length of time between transfusion of the patient and collection of the blood sample and the number of units transfused will influence the effective­ness of this procedure.
   2. Patients must be producing reticulocytes for this method to be successful.

1. **References**
   1. Ottawa Hospital in-house method developed from stated principles.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Revised sections 1.0 & 2.0 * Replaced “red cells” with “whole blood” in section 3.0 * Changed RT.004 to RT.007 in section 6.10 * Revised wording to include “for this method to be successful” in section 8.2 |