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.

1. **Scope and Related Policies**
   1. In the patients who have a high reticulocyte count (10% or higher), the following procedure may be useful in separating the patient's own cells (reticulocytes) from the transfused cells.
   2. 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.

The method chosen will depend upon the volume of the sample and the time frame.

1. **Specimen**

EDTA anticoagulated whole blood preferably less than 72 hours old.

1. **Material**

**Equipment:** Serological centrifuge

**Supplies:** Wintrobe hematocrit Test tubes

Serological pipettes

**Reagents:** N/A

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

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| * 1. Fill several Wintrobe hematocrit tubes with whole blood from EDTA blood sample. |
| * 1. Centrifuge at 1500 g for one hour. |
| * 1. Remove plasma and buffy coat. |
| * 1. Gently remove the top tenth of the red cell column; this should contain predominant­ly reticulocytes. The reactions of these cells in the direct antiglobulin test and in blood grouping tests can be compared with the reactions obtained with cells taken from the bottom layer of the red cell column. See NRT.009- Antigen Typing- Direct and Indirect Agglutination, RT.004 -Direct Antiglobulin Test. |
| * 1. See Procedural Notes 8.1 for examples of results using separated cells. |

1. **Reporting – N/A**
2. **Procedural Notes**
   1. Example of Results using separated cells:

|  |  |  |
| --- | --- | --- |
| Reticulocytes DAT | | |
| Whole Blood | Approx. 10% | ++ |
| Top Layer | Approx. 70% | O |
| Bottom Layer | 1% | ++ |

1. **References**
   1. B.E.E. Croucher, A Seminar On Laboratory Management of Hemolysis p.157, AABB 1979.
2. **Revision History**

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| --- | --- |
| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Revised section 1.0 * Revised and renumbered section 2.0 * Replaced “red cells” with “whole blood” in section 3.0 |