1. **Principle**
	1. Newly formed autologous red cells having a lower specific gravity than transfused red cells may be separated from the transfused population by simple centrifugation in microhematocrit tubes. The autologous red cells will concentrate at the top of a centrifuged microhematocrit capillary tube.
2. **Scope and Related Policies**
	1. To separate the reticulocyte rich portion (RR) and reticulocyte poor portion (RP) from a red cell sample.

* 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
* when it can be demonstrated that the patient reticulocyte count is normal or high
	1. The method chosen for cell separation to isolate autologous cells from transfused cells will depend upon the volume of the sample available and the time frame.
1. **Specimen**

EDTA anticoagulated whole blood preferably less than 24hours old.

1. **Material**

**Equipment:** Microhematocrit equipment

* Centrifuge
* Sealant
* Plain glass microhematocrit capillary tubes (not heparinized)
* Metal file (to cut glass capillary tubes)

**Supplies:** Test tubes – 10 x 75 mm

 2 mL syringe and 23 gauge needle or Pasteur

 pipettes

**Reagents:** 0.9% saline

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

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| * 1. Centrifuge blood sample at 3000 rpm for 10 minutes. Remove plasma as completely as possible without aspirating any red cells.
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| * 1. Thoroughly mix the packed red cells by inversion or by pipetting.
 |
| * 1. Fill four capillary tubes with packed red cells. Seal with putty.
 |
| * 1. Centrifuge in a microhematocrit centrifuge for 15 minutes.
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| * 1. Cut the top 2-5 mm and the bottom 2-5 mm from each of the 4 capillary tubes to obtain the top RR and bottom RP cell populations.
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| * 1. Flush the red cells from the tube segments into appropriately labelled 10x75 mm test tubes using a saline filled syringe and 23-gauge needle or a disposable Pasteur pipette.
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| * 1. Wash the RR and RP twice with 0.9% saline and resuspend to 3% in 0.9% saline.
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| * 1. Test the RR, RP and unseparated red cells in parallel for comparison of DAT and red cell phenotypes. Careful observation of the RR cells for mixed field agglutination is necessary to ensure that the cell separation was effective in isolating autologous red cells.
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1. **Reporting – N/A**
2. **Procedural Notes**
	1. Separation is better when blood samples are obtained 3 or more days after transfusion so that substantial numbers of reticulocytes have time to accumulate.
	2. The packed red cells should be mixed continuously just prior to filling of the microhematocrit tubes.
3. **References**
	1. Judd, WJ ed. Judd’s Methods in Immunohematology, 3rd ed, Bethesda, MD: pg. 174-176.
4. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual
* Revised wording of section 1.0
* Revised wording and renumbered section 2.0
* Replaced “red cells” with “whole blood” in section 3.0
* Added “metal file (to cut glass capillary tubes)” to section 4.0- *Equipment*; Replaced “normal saline” with “0.9% saline” in section 4.0-*Reagents*
* Revised wording to include “10x75mm test tubes” in section 6.6
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