1. **Principle**

Enzyme treated autologous red cells have increased ability to bind cold antibody for removal from plasma.

1. **Scope and Related Policies**
   1. This procedure must not be used for recently transfused (< 3 months) patients; alloantibodies could be adsorbed by the donors’ red cells.
   2. For recently transfused patients use SP.017 - Adsorption of Cold Agglutinins using Rabbit Erythrocyte Stroma – RESt.
2. **Specimen**

2 mL plasma to be adsorbed

3 mL packed autologous cells

1. **Material**

**Equipment:** Block for test tubes

Waterbath/Heating block at 37°C

Refrigerator

Centrifuge

**Supplies:** Test tubes 13 x 100 mm

Serological pipettes

**Reagents:** 0.9% saline

BCA freeze dried papain (reconstitute according to manufacturer’s instruction)

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

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| * 1. Wash red cells 3 or 4 times in saline and divide into three equal 1 ml aliquots in 13 x 100 mm test tubes. |
| * 1. Add 0.5mL of the reconstituted papain to each tube. |
| * 1. Mix and incubate at 37°C for 10 minutes. |
| * 1. Wash the red cells 3 times in normal saline. Centrifuge the last wash for 5 minutes at 3000 rpm and remove as much of the supernatant saline as possible. See Procedural Notes 8.1. |
| * 1. To one tube of the enzyme treated red cells add 2 mL of autologous plasma. |
| * 1. Mix and incubate at 4°C for 30 minutes. |
| * 1. Centrifuge at 3000 rpm for 5 minutes and immediately transfer plasma into a second tube of enzyme treated red cells. |
| * 1. Mix and incubate at 4°C for 30 minutes. |
| * 1. Repeat steps 6.7 – 6.8 for the third tube of enzyme treated red cells. |
| * 1. Following the final adsorption, test the plasma for alloantibody activity. |

1. **Reporting – N/A**
2. **Procedural Notes**
   1. To avoid dilution of the plasma and possible loss of weak alloantibody activity during the adsorption process, it is important to remove as much of the residual saline as possible in Procedure step 6.4. Placing a narrow strip of filter paper into the packed red cells helps remove saline that surrounds the packed cells.
3. **References**
   1. Judd, WJ ed. Judd’s Methods in Immunohematology, 3rd ed, Bethesda, MD: pg. 444-446.
4. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Changed “normal saline” to “0.9 % saline” in section 4.0- *Reagents* * Revised wording of section 6.0 |