1. **Principle**

ZZAP is a combination of Dithiothreitol (DTT) and Papain. ZZAP treatment of red cells removes the autoantibodies coating the patient’s red cells, freeing up antigen sites. ZZAP treated red cells then have increased ability to bind warm autoantibodies for removal from the plasma to allow the detection of any underlying alloantibodies.

1. **Scope and Related Policies**
   1. This procedure must not be used for recently transfused (< 3 months) patients as circulating donor red cells could adsorb alloantibodies.
2. **Specimen**

EDTA anticoagulated whole blood preferably less than 72 hours old.

1. **Material**

**Equipment:** Cell Washer

Serological centrifuge

Block for test tubes

Microscope

Water bath/Heating block at 37°C

**Supplies:** Test tubes – 10 x 75 mm

Serological pipettes

**Reagents:** pH 7.3 phosphate-buffered saline (PBS)

0.2 M DTT (7.7g/250 mL PBS 7.3)

BCA freeze dried papain - reconstituted according to manufacturer’s direction.

0.9% NaCl

**Preparation of ZZAP Reagent**: (Prepare fresh each day of use)

1. Place 2.5 mL of 0.2 M DTT in a test tube.
2. Add 0.5 mL of reconstituted papain solution.
3. Add 2.0 mL of pH 7.3 PBS (must be made fresh).

Volume prepared would be sufficient to treat 2.5 mL of packed red cells.

1. **Quality Control** 
   1. ZZAP treated cells should be nonreactive with anti-k.
2. **Procedure**

**Treatment of Autologous Red Cells For Auto Adsorptions:**

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| * 1. Add two volumes of ZZAP reagent to one volume of patient's packed red cells. Cells do not need to be washed. |
| * 1. Incubate at 37°C for 30 minutes with occasional mixing. |
| * 1. Wash red cells three times with large volumes of 0.9% saline. |
| * 1. Centrifuge last wash at 3000 rpm for at least five minutes and remove as much supernatant as possible. |
| * 1. Perform a direct antiglobulin test (DAT) on the ZZAP treated patient's red cells to determine if IgG has been removed from the cells. |
| * 1. ZZAP treated red cells are ready to use for auto adsorption if the DAT is negative or significantly reduced as compared to the DAT on the untreated red cell sample. |
| * 1. Save a few drops of a 3% saline suspension of ZZAP treated red cells for later testing. |

**Adsorption Technique:**

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| * 1. Divide ZZAP treated red cells into three equal aliquots. |
| * 1. Add an equal volume of the patient's plasma to one aliquot of packed ZZAP treated red cells. Stopper and mix well. |
| * 1. Submerge the tube in a 37°C water bath in a horizontal position incubate for 30 minutes. Gently mix every 10 minutes. |
| * 1. Centrifuge at 3400 rpm for 2-3 minutes and transfer the plasma to the second aliquot of ZZAP treated red cells. Mix. |
| * 1. Repeat step 6.10. |
| * 1. Centrifuge at 3400 rpm for 2-3 minutes and transfer the plasma to the third aliquot of ZZAP treated cells. Mix. |
| * 1. Repeat step 6.10. |
| * 1. Centrifuge at 3400 rpm for 2-3 minutes and transfer the adsorbed plasma to a clean appropriately labelled tube. Three adsorptions are usually sufficient to remove antibody from the plasma in all but the most potent high titre autoantibody. |
| * 1. Test the adsorbed plasma against antibody screening cells and the patient's ZZAP treated red cells (if DAT negative) for antibody activity using an indirect antiglobulin technique. |

1. **Reporting** 
   1. Interpretation of Indirect Antiglobulin Results Against:

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| ANTIBODY SCREEN CELLS | PATIENTS ZZAP  TREATED RBC | INTERPRETATION |
| No Reactivity | No Reactivity | It is unlikely that alloantibody present. |
| Reactivity Against  All Cells | Reactivity | Residual autoantibody present fur­ther adsorption may be required |
| Reactivity Against All Cells | No reactivity | Plasma contains autoantibody within Kell system further autoadsor­ption using Chloroquine or heat-treated red cells indicated. |
| Reactivity Against  One or More Cells | No Reactivity | Plasma contains alloantibody, antibody identification studies required.  2 |

1. **Procedural Notes**

* 1. In addition to antigens normally destroyed by enzymes (M, N, Fy and S) ZZAP treatment of red cells also destroys Kell system antigens with the exception of Km, which is unaffected and Kx which is enhanced.
  2. Hemolysis of red cell samples treated with ZZAP reagent is minimal but may vary according to the age of the sample and degree of autoantibody sensitization.

1. **References**
   1. Judd, WJ ed. Judd’s Methods in Immunohematology, 3rd ed, Bethesda, MD: pg. 461-463.
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual * Changed document number from SP.011 to SP.010 * Revised section 1.0 * Changed wording from “red cells” to “whole blood” in section 3.0 * Added section 5.1 * Revised and renumbered section 6.0 * Revised table in section 7.1 * Updated reference list to include most recent editions |