**RED BLOOD CELL SUSPENSION PREPARATION**

I. **Principle:** - The ratio of antibody to antigen may dramatically affect the sensitivity of agglutination tests. Consistent preparation of 2-5% Red Blood Cell (RBC) suspensions is critical to any agglutination test. Washing the RBCs prior to preparing a 2-5% RBC suspension will remove residual plasma/serum and cellular debris, which may eliminate testing discrepancies.

II. **Equipment and Reagents:**

12 x 75mm test tubes
0.9% Saline
Dispo pipettes
Serofuge

III. **Procedure:**

A. Place 2 or 3 drops of the RBCs to be tested in a test tube that has been labeled with specimen identification (patient’s initials or donor identification number [DIN]).

B. Fill the tube 2/3 to 3/4 full with saline.

1. **Do not** overfill (no closer than 1/2" from the top of the tube) as this may cause splashing and cross-contamination of RBC suspensions.

2. **Do not** contaminate the tip of the saline bottle with RBCs.

C. Centrifuge the tube on high speed (3500 rpm) for 1 min.

D. Decant saline from tube.

E. Repeat washes for the number of times stated in the procedure being performed.

F. Resuspend the RBCs with saline to a 2-5% suspension. Suspension should be a cherry red color. Commercially prepared 2-5% RBC suspensions serve as a good guide for comparison in the preparation of RBC suspensions.

1. If the color of the suspension is too dark, add additional saline to the tube until the suspension color is correct.

2. If the color is too light, centrifuge the tube with the suspension again, for 1 minute and decant the supernatant saline. Resuspend the RBCs with enough saline to produce a cherry red color.
IV. Additional Notes:

A. For most testing, RBCs need only be washed once.

B. Cord blood specimens should be washed a minimum of 3 times prior to testing, in order to remove Wharton’s jelly.

V. References:
Manufacturer’s directions