**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Preparation of red cell suspension**

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| **SOP no.**  | **Effective Date** | **Pages** | **Prepared by**  | **Authorised by**  |
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| **LOCATION** : Red Cell Serology Laboratory |
| **SUBJECT** : Preparation of Red Cell Suspensions |
| **FUNCTION** : To prepare RBC suspension of Appropriate Concentration for a given test. |
| **DISTRIBUTION**: Red Cell Serology Laboratory Master File |

1. **SCOPE & APPLICATION:**

This procedure applies to all testing that requires red cell suspension preparation.

1. **RESPONSIBILITY:**

It is the responsibility of every technician performing a given test to prepare the appropriate red cell suspension. Every morning, the shift duty technician must prepare A, B & O red cell suspension for the day’s use.

1. **MATERIAL REQUIRED:**

**Equipment:**

 Calibrated Centrifuge.

**Reagents:**

 0.9% saline.

**Glassware:**

* + Pasteur pipettes.
	+ Glass Serum tubes.

**Miscellaneous:**

* + Discard box.
	+ 2 plastic beakers.
	+ Rack to hold tubes.

**4. PROCEDURE:**

**Principle:** The ratio of serum to red cells may dramatically affect the sensitivity of agglutination tests. Consistent preparation of either 2 to 5% red cell suspension is critical to any agglutination test.

**Preparation of Pooled Cell Suspension:**

1. Label tubes with A, B, & O groups.
2. Place 1 drop of red cells each from 3 of A group sample tubes or segment into the A labelled tube.
3. Place 1 drop of red cells each from 3 of B group sample tubes or segment into the B labelled tube
4. Place 1 drop of red cells each from 3 of O group sample tubes or segment into the O labelled tube
5. Fill the tube ¾ full with 0.9% saline to resuspend the cells.
6. Centrifuge the tubes for at least 2 to 3 minutes on high speed. Decant the supernatant fluid.
7. Remove any debris or fibrin with the pipette. Add enough saline to produce a cherry red colour comparable to that of the reagent red cell suspension.
8. If the colour is too dark, add additional isotonic saline to the tube until the suspension colour is right.
9. If the colour is too light, repeat steps 6 and 7.
10. Test the pooled cells prepared using the antisera (anti-A, B, AB & D) in use.

Proceed to use the same procedure to prepare cell suspension of particular donor or patient sample for grouping and crossmatching.

**LIMITATIONS:**

Hemolysis of the red blood cells from improper washing may result in false results. A cell suspension that is too heavy or too light may produce false – positive or false negative results.

**Procedure of the preparing pooled reagent red blood cells**

*Pooled A cells* Pool equal quantity of fresh A group cells from anticoagulated sample of three donors. Wash three times with normal saline. Make 2-5% suspension in saline for use.

*Pooled B cells* Pool equal quantity of fresh *B* group cell from anticoagulated sample of three donors. Wash three times with normal saline. Make 2-5% suspension in saline for use.

*Pooled O cells* Pool equal quantity of fresh O group cell from anticoagulated sample of three donors. Wash three times with normal saline. Make 2-5% suspension in saline for use.

**Grading of agglutination**

To record the difference in the strength of reaction. It is necessary to have a system of grading or scoring or reaction which should be uniformly followed.

 4+ - 1 big clump with clear supernatant.

 3+ - 2 /3 big clumps with clear supernatant.

 2+ - many small clumps with clear supernatant

 1+ - many small clumps with turbid supernatant

 W - Granular suspension.

 O - Smooth suspension (Negative)

 I I - Partial/complete hemolysis

**Precautions**

1. Always uses thoroughly cleaned test tubes for the test.
2. While reading the result, shake very gently to dislodge the button.
3. Use correct speed and time of centrifugation to avoid false positive results.
4. **DOCUMENTATION:**
* Enter the donor unit numbers from which pooled cells are prepared in the donor register.
* Record the results of testing with the antisera in use.
* Enter the manufacturer’s name and batch number of the antisera.
1. **References:**
2. Technical manual of American Association of Blood Bank, 15th Edition, 2005.
3. Introduction to Transfusion Medicine; Zarin Bharucha & D.M. Chouhan, 1st Edition, 1990.
4. **END OF DOCUMENT.**