**Lab 4 Rabbit Immunization Experiment**

**Serology and Vaccines**

**1- Preparation of Erythrocytes (human and rabbit) for injection**

Draw human (rabbit) blood into heparinized or EDTA tube and follow the following protocol.

1. Centrifuge whole blood at 500xg for 10 min .
2. Aspirate supernatant (plasma) and add cell wash buffer (normal saline) to erythrocyte pellet.

 **Note: Ensure volume of cell wash buffers double the volume of erythrocyte pellet alone.**

1. Centrifuge erythrocytes at 500xg for 10 min
2. Aspirate supernatant and add cell wash buffer to erythrocyte pellet. Note: Ensure volume of cell wash buffers double the volume of erythrocyte pellet alone.
3. Repeat steps c. and d. two more times for a total of 3 washes of the erythrocytes.

**Note: If erythrocytes appear to be re-suspending, increase the deceleration time of the centrifuge.**

 **Note: DO NOT** store isolated erythrocytes on ice.They are fine at room temperature for (4 – 5) hours or refrigerated for 2 days. They do need to be used on day of isolation.

1. Prepare 5% of washed RBC suspension.
2. Inject animals with 0,5 ml of 5% human RBC suspension intra peritoneal .
3. The first, second and third booster dose given subcutaneous in different sit into the back of the rabbit

**2- Preparation of Serum (human and rabbit) for injection**

1. Prepare serum as previously mentioned.
2. Dilute serum to 1/5 with normal saline.

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| **Injection Schedule** |
| **Ag type** | **Dose** | **Injection route** | **Volume of dose** | **Conc. of dose** |
| R.B.Cs(human or rabbit) | 0 | IP | 2 ml | 5% |
| R.B.Cs(human or rabbit) | 1 | Sc | 2 ml | 5% |
| R.B.Cs(human or rabbit) | 2 | Sc | 2 ml | 5% |
| R.B.Cs(human or rabbit) | 3 | Sc | 2 ml | 5% |

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| **Injection Schedule** |
| **Ag type** | **Dose** | **Injection route** | **Volume of dose** | **Conc. of dose** |
| Serum(human or rabbit) | 0 | IP | 0.5 ml | 0.2-0.5% |
| Serum (human or rabbit) | 1 | Sc | 0.5 ml | 0.2-0.5% |
| Serum (human or rabbit) | 2 | Sc | 0.5 ml | 0.2-0.5% |
| Serum (human or rabbit) | 3 | Sc | 0.5 ml | 0.2-0.5% |

**Collection of blood:**

Ten days after the completion of last booster injection animal bled from the marginal ear vein or by heart puncture to perform the immunological tests to detect Abs formation in rabbit serum.