**Haemoglobin and Anemia**

1. **Hemoglobin Estimation:**

|  |  |  |
| --- | --- | --- |
| **Blank** | **Test** |  |
| 2 ml | 2 ml | **Hemoglobin reagent** |
| \_\_\_\_\_ | 0.01 ml ( 10µl) | **Blood sample** |
| Mix, allow to stand at room temperature for 3 min and read the absorbance at 540 nm against hemoglobin reagent | | |

Hb conc (g/dl) = 29.4 x Abs of test

1. **Qualitative determination of hemoglobin S (HbS) in blood**

|  |  |
| --- | --- |
| **Reagent** | **Volume** |
| Sickling solution | 2 ml |
| Patient sample (whole blood) | 0.02 ml (20 μl) |
| **Mix by inversion and allow stand at room temperature for 5 to 10 min** | |
| **Read the test by holding the test tube approximately 3 cm in front of a lined scale on the card.** | |

1. **Glucose 6 Phosphate determination**

|  |  |
| --- | --- |
| **Reagent** | **Volume** |
| G6PDH Buffer | 3 ml |
| NADP reagent | 100 μl |
| Hemolysate | 50 μl |
| **Mix and incubate for 5 min at 25°C, the add** | |
| G6PDH Substrate | 50 μl |
| **Mix and read absorbance every min for 3 min against distilled water and calculate ΔA/min** | |

|  |  |  |  |
| --- | --- | --- | --- |
| DA/min=[(A3-A2)+(A2-A1)]/2 | Abs 340 nm | | Time |
|  |  | A1 | 1 min |
|  | A2 | 2 min |
|  | A3 | 3 min |

G6PD Activity in mU/**erythrocytes/ml of blood** ( P )= ΔA/min x 30868

***Note: If the erythrocytes count per ml of blood is 5 X 109***

* G6PD activity in mU/ **109 cells** = P/5