**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***

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