

## **Experiment no.: 7.**

**Experiment name:.** Determination of **HEMOGLOBIN** in the blood serum.

### **The aim of the Experiment:**

Determination of **HEMOGLOBIN TOTAL** using *Colorimetric method* ENDPOINT

### **Equipment and materials used in the Experiment:**

- Photometer or colorimeter capable of measuring absorbance at  $540 \pm 20$  nm.
- – 250 mL volumetric, calibrated, glass flask.
- – Pipettes to measure reagent and samples.

**R1 Drabkin's reagent (50x).** Modified. Potassium ferricyanide 30 mmol/L, potassium cyanide 38 mmol/L, potassium hydrogen phosphate 50 mmol/L, surfactant 2.5% (w/v). **Xn**

**Hemoglobin standard.** Hemoglobin 12 g/dL (7.5 mmol/L). Bovine origin. Concentration value is traceable to Standard Reference Material CRM 522.

## **REAGENT PREPARATION**

### **Working reagent**

By automatic pipette withdraw 5 mL of **R1** and deliver the contents into a 250 mL volumetric calibrated flask letting the flow to slide along the neck to minimize foaming. Add distilled water to the mark, cap, and mix by inversion. Stable for at least 6 months at 15- 25 °C when stored in a tightly closed brown borosilicate glass bottle. Discard if reagent becomes darkened or discolored.

### **Property of the machine:**

Normal UV-Vis spectrophotometer:

### **Machine usage:**

- 7- Wavelength set up step.
- 8- Blank against the solvent solution using a proper cuvette.
- 9- Reach O.D.

### Experiment procedure or protocol:

#### 1. PROCEDURE

1. Pipette into labelled tubes:

TUBES	Blank	Sample	CAL. Standard
Working reagent Sample CAL.Standard	2.5 mL — —	2.5 mL 10 µL —	2.5 mL — 10 µL

2. Mix and let the tubes stand 3 minutes at room temperature.
3. Read the absorbance (A) of the samples and the standard at

540 nm against the reagent blank.

The color is stable for several hours. Storage for periods over 6 hours should be kept at 2-8 °C.

#### CALCULATIONS

With Standard:

$A_{\text{Sample}} / A_{\text{Standard}} \times C_{\text{Standard}} = \text{g/dL total haemoglobin}$

With Factor:  $A_{\text{Sample}} \times 36.8 = C_{\text{Sample}} (\text{g/dL total hemoglobin})$

If results are to be expressed as SI units apply:  $\text{g/dL} \times 0.621 = \text{mmol/L}$

### Experiment data and results:

men	13.5 - 18.0 g/dL (8.4 - 11.2 mmol/L)
Women	11.5 - 16.5 g/dL (7.1 - 10.2 mmol/L)
Newborns (cor blood)	13.6 - 19.6 g/dL (8.4 - 12.2 mmol/L)
Infants, 6 months	12.8 - 16.0 g/dL (8.0 - 10.0 mmol/L)
Infants, 1 year	11.0 - 13.0 g/dL (6.8 - 8.1 mmol/L)
Children, 14 years	11.5 - 14.8 g/dL (7.1 - 9.2 mmol/L)

### Conclusion:

- What is the role of this experiment?
- How does its deficiency affect the health?
- What are the normal level values?
- How can you determine its quantity on the blood?
- Discuss the methodology?