**Experiment no.: 5.**

**Experiment name:** Determination of **GOT/GPT** in the blood serum.

**The aim of the Experiment:**

Determination of **GOT/GPT** in the blood using REITMAN-FRANKEL *Colorimetric method* ENDPOINT

**PRINCIPLE**

Aspartate aminotransferase (GOT) catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxalacetate.

L-Aspartate + 2-Oxoglutarate L-Glutamate + Oxalacetate

Alanine aminotransferase (GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate.

L-Alanine + 2-Oxoglutarate Glutamate + Pyruvate

The transaminase activity is proportional to the amount of oxalacetate or pyruvate formed over a definite period of time and is measured by the reaction with 2,4-dinitrophenylhydrazine (DNPH) and measurement of the color formed in an alkaline solution[1].

**Equipment and martials used in the Experiment:**

- − Photometer for measurements at 505 nm ± 15 nm.
- − Thermostatic water bath set at 37 ºC (± 1 ºC).
- − Stopwatch.
- − Pipettes of 5.0 mL, 1.0 mL and 0.1 mL.
- − Glass tubes.

**REAGENT COMPOSITION**

**GOT substrate**. Phosphate buffer 100 mmol/L pH 7.4,

- R1a L-aspartate 200 mmol/L, ketoglutarate 2 mmol/L.
- **R1bGPT substrate**. Phosphate buffer 150 mmol/L pH 7.4, L-alanine 200 mmol/L, ketoglutarate 2 mmol/L.
- **R2 DNPH.** 2,4-Dinitrophenylhydrazine 1 mmol/L. Color developer. **C R:34/35**
- **R3 4N NaOH** (10x). Sodium hydroxide 4 mol/L. **C R:34/35 Pyruvic standard.** 1.8 mmol/L. Secondary standard.
- **Cal Pyruvic standard.** 1.8 mmol/L. Secondary standard.

- **REAGENT PREPARATION**
- The substrates, standard and color developer are ready-to-use.

- **Working 0.4 N NaOH solution.** By means of a funnel pour the contents of the 10x concentrate 4N NaOH preparation into a 2- liter volumetric flask, rinse the bottle with some volumes of distilled water, complete to the mark and mix. The solution warms up. Let stand till reaches room temperature and complete to the volume. Mix again and store in a well capped polyethylene bottle at room temperature.

**Property of the machine:**

Normal UV-Vis spectrophotometer:

**Machine usage:**

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

**Experiment procedure or protocol:**

1. **PROCEDURE**
    1. Bring reagents and samples at room temperature.
    2. Pipette into labelled tubes:

| TUBES | Blank | GOT | GPT |
|---|---|---|---|
| GOT substrate GPT substrate | 0.5 mL - | 0.5 mL - | - 0.5 mL |
| Warm to 37 ºC into the bath for 5 min. Add: | | | |
| Serum | - | 100 µL | 100 µL |
| Mix. Return to bath at 37 ºC for: **60 min. 30 min.** Add: | | | |
| DNPH | 0.5 mL | 0.5 mL | 0.5 mL |
| Mix. Stand for 20 min. at room temperature. Add: | | | |
| NaOH 0.4 N | 5.0 mL | 5.0 mL | 5.0 mL |
| Invert to mix. Stand for 5 min. at room temperature.<br><br>3. Read the absorbances (A) of the samples against a water blank (Note 1).<br><br>The color is stable for at least 1 hour. | | | |

**Experiment data and results:**

| Serum, plasma<br><br>**REFERENCE VALUES** | | |
|---|---|---|
| Adults | 37 ºC | up to 40 U/L (0.67 μkat/L) |
| | 30 ºC | up to 25 U/L (0.42 μkat/L) |

**Conclusion:**

- Where is GOT synthesized from?
- How does it affect the health?
- What are the normal level values?
- How can you determine its quantity on the blood?
- Discuss the methodology?