

**Comenius University in Bratislava, Faculty of Medicine**  
**Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry**

---

LABORATORY PROTOCOL GM-WS - 9<sup>th</sup> seminar

**Determination of TAG, total cholesterol and HDL in blood serum**

|              |       |
|--------------|-------|
| Name, group: | Date: |
|--------------|-------|

Principle:

Determination of total serum cholesterol: Hydrolysis of cholesterol catalyzed by enzyme *cholesterolesterase* produces free cholesterol. Another enzyme *cholesteroloxidase* oxidizes free cholesterol and one of the products is hydrogen peroxide. Hydrogen peroxide reacts with 4-aminophenazone and phenol thus producing red-coloured product suitable for spectrophotometrical measurement.

Determination of HDL cholesterol in serum: Before determination of HDL lipoproteins concentration, other lipoprotein particles (LDL and VLDL) have to be removed using mixture containing wolframic acid and magnesium chloride. Precipitated lipoproteins are removed by centrifugation and HDL particles remain in the solution. Cholesterol of HDL particles can be determined using the same method we use for determination of total cholesterol.

Determination of serum triacylglycerols

Concentration of triacylglycerols in blood serum is determined through the determination of amount of glycerol released by their hydrolysis. Oxidation of glycerol leads to production of H<sub>2</sub>O<sub>2</sub> which reacts with 4-aminophenazone to give colored complex.

Procedure:

Determination of total cholesterol in serum - procedure:

|          | TCH <sub>1</sub> | TCH <sub>2</sub> | standard       | reference sample |
|----------|------------------|------------------|----------------|------------------|
| serum 1  | <b>0.01 ml</b>   | ---              | ---            | ---              |
| serum 2  | ---              | <b>0.01 ml</b>   | ---            | ---              |
| standard | ---              | ---              | <b>0.01 ml</b> | ---              |
| water    | ---              | ---              | ---            | <b>0.01 ml</b>   |
| reagent  | 1.0 ml           | 1.0 ml           | 1.0 ml         | 1.0 ml           |

We let the samples stand for 20 minutes and measure absorbance at 510 nm.

Determination of HDL cholesterol in serum - procedure:

|             | HDL <sub>1</sub> | HDL <sub>2</sub> | reference sample |
|-------------|------------------|------------------|------------------|
| serum HDL 1 | 0.1 ml           | ---              | ---              |
| serum HDL 2 | ---              | 0.1 ml           | ---              |
| water       | ---              | ---              | 0.1 ml           |
| reagent     | 1.0 ml           | 1.0 ml           | 1.0 ml           |

We let the samples stand for 20 minutes and measure absorbance at 510 nm.

Determination of TAG

| sample  | TAG 1  | TAG 2  | reference sample |
|---------|--------|--------|------------------|
| serum 1 | 0.1 ml | ---    | ---              |
| serum 2 | ---    | 0.1 ml | ---              |
| water   | ---    | ---    | 0.1 ml           |
| reagent | 2.0 ml | 2.0 ml | 2.0 ml           |

We let the samples stand for 10 minutes and measure absorbance at 510 nm.

Calculation:

Total cholesterol:

|                            | TCH <sub>1</sub> | TCH <sub>2</sub> | standard |
|----------------------------|------------------|------------------|----------|
| absorbance                 |                  |                  |          |
| total cholesterol (mmol/l) |                  |                  | 5.0      |

HDL cholesterol:

|   |                  |                  |
|---|------------------|------------------|
| sample  | HDL <sub>1</sub> | HDL <sub>2</sub> |
| absorbance                                    |                  |                  |
| HDL cholesterol (mmol/l)                      |                  |                  |
| correction for standard and dilution (x 3/10) |                  |                  |

TAG

|                           |       |       |
|---------------------------|-------|-------|
| sample                    | TAG 1 | TAG 2 |
| absorbance                |       |       |
| triacylglycerols (mmol/l) |       |       |

Note! - We calculate concentration of HDL cholesterol using standard from total cholesterol measurement. Since we used **only 0.01 ml** of the standard and the volume of the samples was 0.1 ml (10x higher), we have to correct the measured concentration of HDL **by dividing it by 10**. Moreover for this determination the sera were diluted **1:3** (we multiply the concentration by 3)

LDL cholesterol:

LDL cholesterol can be calculated using Friedewald equation:

$$\text{LDL} = \text{total cholesterol} - (\text{TAG} / 2.2 + \text{HDL})$$

(TAG must not be higher than 4.6 mmol/l, otherwise the LDL calculation would be imprecise.)

Atherogenic index (AI):

$$\text{AI} = (\text{total cholesterol} - \text{HDL}) / \text{HDL}$$

(Values lower than 4.85 mean low risk, values over 4.85 mean high risk of atherosclerosis.)

Results:

|                   | Patient 1 | Patient 2 |
|-------------------|-----------|-----------|
| TAG               |           |           |
| total cholesterol |           |           |
| LDL               |           |           |
| HDL               |           |           |
| atherogenic index |           |           |

Reference values:

|                   | gender | physiological values |
|-------------------|--------|----------------------|
| TAG               | M + F  | 0.45 – 1.7 mmol/l    |
| total cholesterol | M + F  | 3.1 – 5.0 mmol/l     |
| LDL               | M + F  | < 3.25 mmol/l        |
| HDL               | M      | 1.0 – 2.1 mmol/l     |
|                   | F      | 1.2 – 2.7 mmol/l     |

Conclusion:

**Literature for next week:**

- General reactions of amino acid metabolism (deamination, transamination, decarboxylation).
- Enzymes and importance of pyridoxalphosphate (PLP) in amino acid metabolism.
- Lippincott's: Chapter 19, Part V - VII. Formulas: Fig. 19.11; 19.14; 19.17; 19.18.
- Practical exercises in biochemistry (Asklepios, 1993) – Chapter 10 - Theoretical part.