

Instructions for the practical lesson on biochemistry

Topic: Biochemical examination of cerebrospinal fluid

Task 1: Qualitative estimation of protein in CSF (Pandy's test)

Reagents:

1. Phenol 100 g/l
2. Sample of CSF (infectious material)
3. Sample of serum (infectious material)



Procedure:

Measure 0.5 ml of phenol solution onto a watch glass. Add a drop of the CSF sample to one side of the spot of phenol solution and a drop of serum (positive control) to the other side. Wait 3 minutes and evaluate the degree of turbidity. In case of a positive result turbidity develops after addition of CSF. Use the signs + to +++ to evaluate the degree of turbidity. The serum sample, whose protein concentration is cca 200-times higher compared to the CSF, serves purely as a positive control in this test. Watch the turbidity against a dark background.

Task 2: Quantitative estimation of protein in CSF

Reagents:

A commercial kit Celková bílkovina 600 M made by SKALAB Svitavy is used for analysis.

1. Buffer
 - Sodium benzoate 6.94 mmol/l
 - Succinic acid 100.0 mmol/l
 - Sodium molybdate 0.12 mmol/l
 - Sodium oxalate 2.09 mmol/l
 - Detergents and stabilizers
2. Chromogen
 - Pyrogallol red 0.14 mmol/l
 - Stabilizers
3. Standard protein solution 1.0 g/l
4. Unknown sample of CSF

Procedure:

Prepare and mark 3 test tubes. Measure the solutions according to the table:

| Measure in ml | Test tube 1 CSF sample | Test tube 2 Standard | Test tube 3 Blank |
|-----------------|---------------------------|-------------------------|----------------------|
| CSF sample | 0.02 | – | – |
| Standard | – | 0.02 | – |
| Deionized water | – | – | 0.02 |
| Buffer | 0.5 | 0.5 | 0.5 |
| Chromogen | 0.5 | 0.5 | 0.5 |

Mix the tubes and incubate 5 minutes at the room temperature. Measure the absorbances of sample and standard against the blank in 1 cm cuvette at 600 nm.

Calculation:

$$\text{Sp-Total protein (g/l)} = \frac{A_{\text{CSF}}}{A_{\text{standard}}} \times \text{standard concentration}$$

Task 3: Estimation of albumin concentration in CSF and serum**Reagents:**

A commercial kit Albumin Biosystems is used for analysis.

1. Reagent

| | |
|-----------------------|--------------|
| Bromocresol green | 0.27 mmol/l |
| Acetate buffer pH 4.1 | 100.0 mmol/l |
| Detergent | |

2. Standard solution of albumin (infectious material) 50 g/l

3. Unknown CSF sample (infectious material)

4. Unknown serum sample (infectious material)

Procedure:

Prepare and mark 4 test tubes. Measure the solutions according to the table:

| Measure in ml | Test tube 1 CSF sample | Test tube 2 Serum sample | Test tube 3 Standard | Test tube 4 Blank |
|-----------------|---------------------------|-----------------------------|-------------------------|----------------------|
| Reagent | 1.0 | 1.0 | 1.0 | 1.0 |
| CSF sample | 0.1 | – | – | – |
| Serum sample | – | 0.01 | – | – |
| Standard | – | – | 0.01 | – |
| Deionized water | – | – | – | 0.01 |

Mix the tubes and incubate 1-5 minutes at the room temperature.
Measure the absorbances of samples and standard against the blank in 1 cm cuvette at 610 nm.

Calculation:**a) Concentration of albumin in CSF:**

$$\text{Sp-Albumin (g/l)} = \frac{A_{\text{CSF}}}{A_{\text{standard}} \times 10} \times \text{standard concentration}$$

b) Concentration of albumin in serum:

$$\text{S-Albumin (g/l)} = \frac{A_{\text{Serum}}}{A_{\text{standard}}} \times \text{standard concentration}$$

c) Albumin quotient:

$$Q_{\text{alb}} = \frac{\text{Albumin in CSF}}{\text{Albumin in serum}}$$

Task 4: Estimation of glucose concentration in CSF and serum**Reagents:**

Kit Bio-La-Test Glukosa Liquid 1000 made by Erba-Lachema a.s. is employed for the analysis.



1. Working solution OGTT:

| | |
|------------------------|----------------|
| glucose oxidase | ≥ 166.0 μkat/l |
| peroxidase | ≥ 16.0 μkat/l |
| 3-methylphenol | 10.0 mmol/l |
| 4-aminoantipyrine | 1.0 mmol/l |
| phosphate buffer, pH 8 | 140.0 mmol/l |
2. Standard solution of glucose 10 mmol/l
3. Unknown CSF sample (infectious material)
4. Unknown serum sample (infectious material)

Procedure:

Prepare and mark 4 test tubes. Measure the solutions according to the table:

| Measure in ml | Test tube 1 CSF sample | Test tube 2 Serum sample | Test tube 3 Standard | Test tube 4 Blank |
|------------------|---------------------------|-----------------------------|-------------------------|----------------------|
| Working solution | 1.00 | 1.00 | 1.00 | 1.00 |
| CSF sample | 0.01 | – | – | – |
| Serum sample | – | 0.01 | – | – |
| Standard | – | – | 0.01 | – |
| Deionized water | – | – | – | 0.01 |

Mix the tubes and incubate 30 minutes at room temperature in dark. Then measure the absorbances of CSF, serum and standard against the blank at 500 nm.

Calculation:

a) Concentration of glucose in CSF:

$$\text{Sp-Glucose (mmol/l)} = \frac{A_{\text{CSF}}}{A_{\text{standard}}} \times \text{standard concentration}$$

b) Concentration of glucose in serum:

$$\text{fS-Glucose (mmol/l)} = \frac{A_{\text{Serum}}}{A_{\text{standard}}} \times \text{standard concentration}$$

c) Glucose quotient:

$$Q_{\text{glu}} = \frac{\text{Glucose in CSF (mmol/l)}}{\text{Glucose in serum (mmol/l)}}$$

Task 5: Spectrophotometry of CSF

Procedure and evaluation:

Measure spectra of three CSF samples (prepared in cuvettes at the spectrophotometer) in the wavelength range 350-600 nm against distilled water. Make a sketch of each spectrum into your report and notice any eventual absorption maxima. Conclude which (if any) of the blood pigments (oxyhemoglobin, methemoglobin, bilirubin) are present in the sample and whether the finding is an evidence of a subarachnoid hemorrhage.

Task 6: Evaluation of isoelectrophoreograms of CSF and serum

Procedure and evaluation:

Make a sketch of isoelectrophoreograms of cerebrospinal fluid and serum from two patients. Try to determine which of the possible five types these results represent and include an example of a relevant pathological condition.