

## **The concept of the subject spec. immunologist. methods, winter semester 2023, 215 lab, A36**

Time schedule of block exercises from the subject **Bi9250cen Special Immunological Methods – Practical Course; 6 students**

Things with you: slippers and lab coat, protocols, mobile phone for photography, flash drive, snack

In the practical exercise we will deal with tests of the following methods of IS activity

(a) invertebrates

(b) vertebrates

**Methods used: blood collection and processing (blood differential), hemolymph collection; ELISA (enzyme immunoassay) - 1. determination of the presence of antibodies against B.b.s.l. (*Borrelia afzelii, garinii, sensu stricto*), 2. determination of the presence of antibodies against COVID-19 – just test; lymphocyte activity and cytotoxicity assay; rapid immunochromatographic method for determining the presence of Ag or Ab for various detections; liver enzyme ALT assay; observation of hemolymph cells (insect hemocytes); phagocytosis activity assay using phagocytosed particles in insect hemocytes; preparation by spreading the tick intestines; demonstration of the presence of erythrocyte antigens in human saliva - indirect haemagglutination (HIT method).**

Term: Wed.-Fri. 10. – 13.1. from 8.30 am to cca 15-16 pm

Tuesday 8:30

**Instructions for the day of the exercise 1. blood smear, staining and preparation of serum 2. preparation of plates for ELISA, preparation of antigen for plates 3. determination of liver enzyme ALT 4. immunochromatographic test of HIV, drawing for other immunographic tests.**

In detail: gradual blood sampling from the finger, ELISA - Et OH, washing of plates, determination of conc. antigen, Ag dilution, calibration curve and plate pipetting; meanwhile smear stains and blood differential; ALT determination, drawing for immunochromatic test, blood processing for serum

**Tools:**

**ELISA:** prepared A free plates, Ag already sonicated, albumin, calibration reagents, binding solution, refrigerator, et-OH

**Transfer of instructions and protocols to the immunochromatographic test-roulette**

**ALT:** serum, ELISA reader with 340 nm filter, epins, plate

Blood collection: gloves, stand, spread glasses, disinfection, needles, staining kit-Leukodif, pipettes, tips, waste container, stands, ependorfs, lottery papers

Wednesday 8:30

**Instruction: 1. immunochromatographic tests 2. lymphocyte activity test 3. hemolymph collection from larvae**

**In detail:** 1. immunochromatographic tests 2. lymphocyte activity test 3. hemolymph collection from wax moth larvae, cultivation with starch particles and monitoring of phagocytic abilities of hemolymph, staining of glasses and examination of hemocytes under the microscope, counting FI and % F, blood differential; 4. preparation of the preparation from the gut of the tick 5. preparation of the plates for ELISA - continued: removal of Ag and washing, pipetting of the binding solution with casein, incubation with casein, washing of the plates

**Tools:**

Phagocytosis: Leukodif, starch grain solution, larvae, smear slides, thermostat, small epins, tweezers, scissors, syringes and needles

Pipettes, tips, waste container, stands, ependorfs, thermal bath, small thermostat  
ELISA: blocking-binding solution with casein, washing solution, colloidal silver 50 ppm, washer

Thursday 8:30

**Instruction - HIT method**

**In more detail:** HIT method - detection of the presence of AB0 blood system antigens in human saliva

**Tools:** Commercial diagnostics (EXBIO Olomouc) anti-A (IgM) monoclonal, anti-B (IgM) monoclonal, anti-H monoclonal; influx of diagnostic erythrocytes A, B, 0; bromelain, buffered saline 0.85% (0.15 mmol / l) NaCl, plates for reading results, water bath at 100 ° C, centrifuge, test tubes, tube racks, pipettes, tips, gloves, ajatin solution, vials for waste, markers

Friday: 8:30

**Instructions: 1. ELISA alone**

dilution of sera, distribution of samples per plate, preparation of diluted sera: incubation of the plate with sera (dilute conjugate), washing of the plate, application of conjugate, incubation with conjugate (prepare substrate solution with OPD), washing and application of substrate solution, incubation with substrate solution, stopping the reaction with H<sub>2</sub>SO<sub>4</sub>, measurement on ELISA-reader. In the meantime completion of methods, control of results.

**Tools:**

**ELISA:** blocking solution, washing solution, washer, conjugate, substrate solution, substrate, H<sub>2</sub>SO<sub>4</sub>, ELISA-reader at 492 nm  
Pipettes, tips, stands, ependorfs

**overview of methodologies:**

**Blood collection and processing, serum preparation, blood differential determination**

**ELISA - determination of the presence of antibodies against B.b.s.l.**

**ELISA - determination of the presence of antibodies against COVID-19 – just test**

**Turbidimetric determination of the presence of ALT enzyme**

**Lymphocyte activity and cytotoxicity test, ATP test**

**Liver cell activity test, ALT test**

**Observation of hemolymph cells (insect hemocytes), cell staining**

**Phagocytosis activity test using starch grains in insect phagocytes, cell staining**

**Preparation of the gut from a tick**

**Demonstration of the presence of erythrocyte antigens in human saliva (HIT)**

**Rapid qualitative test to determine the presence of HIV**

**Furthermore, a selection of other tests**

**Rapid immunochromatographic method for determining the presence of hemoglobin in stool - individual determination**

**Rapid immunochromatographic method for determining the presence of Helicobacter pylori in faeces**

**Rapid test for the determination of Streptococcus A (pyogenes) in the throat**

**Rapid test for the determination of Streptococcus pneumoniae in urine**

Change of programme is reserved