

The concept of the subject spec. immunologist. methods, winter semester 2021
Time schedule of block exercises from the subject **Bi9250cen Special Immunological Methods – Practical Course; 6 students**

Aids with you: slippers and 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 (immunoenzyme analysis) - 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; lymphocyte activity and cytotoxicity assay; rapid immunochromatographic method for determining the presence of Ag or Ab; 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: út.-pá. 8. – 11.2. 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

pá 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 substr. solution, incubation with substrate, stopping the reaction with H₂SO₄, measurement on ELISA-reader, in the meantime: blood differential, FI,% phagocytosis. Completion of methods, control of results

Tools:

ELISA: blocking solution, washing solution, washer, conjugate, substrate solution, substrate, H₂SO₄, ELISA-reader at 492 nm
Pipettes, tips, stands, ependorfs

verview 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

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 preparation from the gut of 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