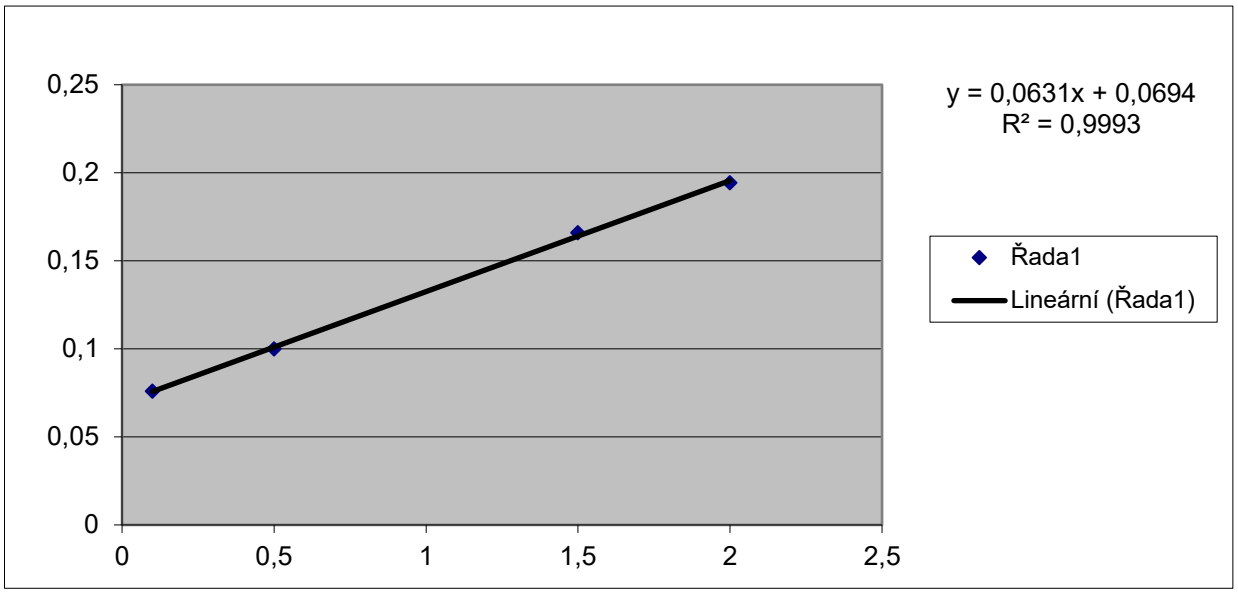
ELISA protocol

The methodology consists of four parts: 1. Determination of antigen concentration
2. Binding of antigen to plate
3. Optimization of ELISA method
4. Examination of ELISA samples
5. Statistical evaluation - determination of the limit of positivity

1. Determination of antigen concentration

Determination of antigen concentration using a calibration curve using different dilutions of albumin. To determine the antigen concentration it is necessary to:

1. Prepare 5 different albumin concentrations: 2 mg / ml; 1.5 mg / ml; 1 mg / ml; 0.5 mg / ml; 0.1 mg / ml.
2. Mix 5 µl of the sample with 25 µl of Reagent A solution and 200 µl of Reagent B in triplicate on a microplate. After approx. 20 min. the absorbance at 700 nm is measured.
3. Create a calibration curve from the albumin values and calculate the protein concentration.



průměrné koncentrace (mg/ml)	**SS**	**A**	**G**
	1,139989	0,484945	0,727945

The antigen concentration of B. b. Sensu stricto strains is 1.139989 mg / ml, B. afzelii is 0.484945 mg / ml, and B. garinii is 0.727945 mg / ml.

Another example of CALIBRATION

Results:

determination of antigen concentration

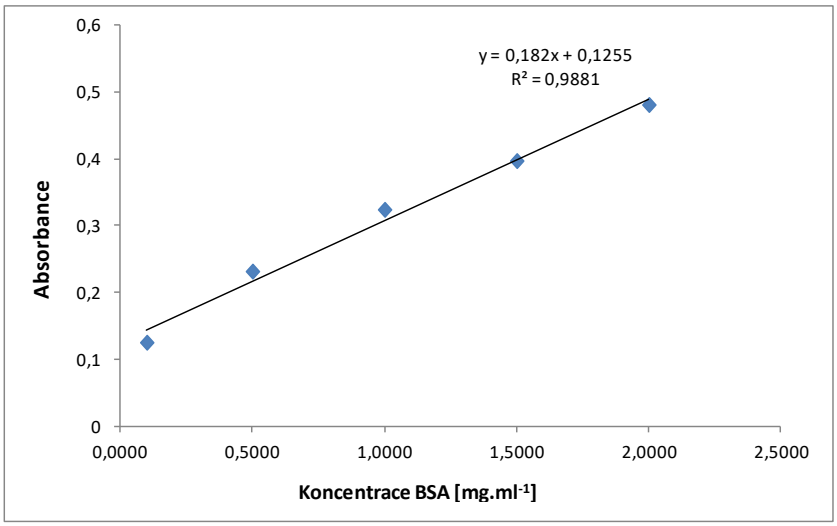


Figure 1: Concentration dependence of absorbance value on bovine serum albumin concentration.

The calibration equation was obtained from Figure 1: y = 0.182x + 0.1255
 ie Y = 0.182 x c protein + 0.1255. From this equation, the concentrations of sonicated cellular antigen of uniform borrelia species can be calculated.
 c protein = (Y - 0.1255) / 0.182 [mg.ml-1]
 Attention !!! The graph must have A on the X axis, c on the Y axis
Calculation: the antigen concentration is 5850µg / ml, ie 5.585mg / ml
We need 10 plates (100ml) with a concentration of 3µg / ml
How many µl do I take from the antigen?

3 µg …… .100ml
5850 µg …… xml
Indirect proportion
5850: 3 = 100: x
X = (5850: 3) .100
X = 51.28 µl = 0.051ml of antigen and make up to 100ml

2. Procedure for binding antigen to plate

After determining the antigen concentration, the next step is to bind the antigen to the plate. The plate should be handled very carefully at all times and should not touch the underside of the wells.

1. Pipette 200 µl of ethanol (70%) into each well of the microplate. The plate is then covered with a lid and allowed to stand still on a horizontal surface at room temperature for about 2 hours.

2. After the specified time, the ethanol is aspirated and the wells are washed 3 times with distilled water (200 µl per well). Residual liquid from the wells should be removed by gently striking clean filter paper.

3. Dilute the antigen to 2-3 □g / ml in the binding solution. 100 ml of the diluted antigen is pipetted into each well.

4. The plate thus prepared is covered with a lid at this stage and left overnight in the refrigerator (at 4 ° C). It is necessary to maintain its horizontal position again.

5. Pipette wells the next day and rinse 3 times with wash solution (200 µl per well), shake and allow to dry.

6. Now it is necessary to saturate the remaining area on the plastic, where the antigen is not bound. Pipette 100 µl of binding solution with dissolved 3% casein into each well. (The buffer must be at room temperature to completely dissolve the casein in the solution.) Now allow the plate to stand at room temperature for about 2 hours.

7. Aspirate the wells and wash 3 times with 200 µl of Wash Solution, then shake the plate again and allow to dry.

8. The plates are now ready for ELISA. At this stage, the plates can also be stored in the refrigerator for 2 months (it is important to prevent the access of moisture).3.

1. First, the sera to be examined should be diluted to an optimal concentration with a blocking solution with 0.3% casein. The diluted sera are pipetted at 100 µl per well.

2. The plate with diluted sera and well-sealed lid is now placed in the thermostat and incubated at 37 ° C for 1 hour.

3. After the set time has elapsed, the plate is removed from the thermostat, the well solutions are aspirated, and the wells are washed at least 3 times with wash solution (200 µl per well). Gently tap to remove residual liquid from the plate into a clean filter paper.

4. Dispense 100 ml of conjugate diluted with blocking solution into all wells and incubate the whole plate at 37 ° C for 1 hour.

5. After incubation in the thermostat, the well contents are aspirated, washed again at least 3 times with wash solution (200 µl per well) and the whole plate is shaken.

6. Now it is necessary to prepare a substrate solution with chromogen and substrate at 100 µl per well by mixing 15 ml of substrate solution with 7.5 mg OPD and 7.5 µl H2O2.

7. Place the plate and the lid in the dark and incubate in a horizontal position at room temperature for about 20 minutes.

8. After the determined time, the reaction is stopped by adding 1-2 M H2SO4 (50 µl per well). The best way to do this is to measure at 492 nm on an ELISA reader.

ELISA results - measurement of samples:

ELISA results - sample measurement:

Duplicates were pipetted into each measurement plate: blank, negative control (NK), positive control (PK) and specific samples of individual sera. For graphical representation of ELISA outputs, a blank (A-B) was subtracted from the absolute value of the measured absorbance of each sample.

You need to create a table of results and graphs:

Graphical representation of recalculated absorbance values for IgM and IgG sera: