Borrelia IgM ELISA Kit

Catalog Number KA1468

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The kit is intended for a detection of IgM antibodies to the pathogenic borrelia strains (B. afzelii, B. garinii and B. burgdorferi sensu stricto) in human serum or plasma, cerebrospinal fluid and synovial fluid and for estimation of the intrathecal antibody production.

Background

The detection of antibodies is one of the laboratory tests that help to diagnose Lyme disease (LD). The diagnosis of LD is based on the combination of clinical examination and laboratory testing. Anti-borrelia IgM antibodies are detectable 3 weeks after infection with its maximum during the sixth week. Subsequently, the titre of IgM antibodies decreases and the IgG antibodies prevail. The detection of anti-borrelia antibodies is very important at the early stage of the disease since the typical symptoms are present only in a certain proportion of patients (e.g. erythema migrans is present in 50% of patients). The clinical symptoms of LD are similar to the symptoms in other diseases, therefore the serology is also of use in differential diagnosis of neuroinfections, arthropathies, carditis and skin diseases.
During the interpretation of the test results it has to be taken into account that the titres of antibodies at early stage of the disease is rather low and it can be influenced by the administration of antibiotics. Seronegativity in a small percentage of patients and the crossreactivity of antibodies elevated due to the other diseases has to be considered. Therefore, it is necessary to compare the test results with the clinical data (adapted from the text at the web page of the Czech National Reference Laboratory for Lyme disease).

Principle of the Assay

Borrelia IgM ELISA assay is a solid-phase immunoanalytical test. The polystyrene strips are coated with the mixture of recombinant borrelia antigens. Anti-borrelia antibodies in serum samples bind to the immobilized antigens. The serum antibodies that do not bind are washed away and those that formed complexes with the antigens are later on recognized by animal anti-human IgM antibodies labelled with horseradish peroxidase. The presence of labelled antibodies is revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and the mild change in colour, if present, may be attributed to the reaction background.

✓ Flow Chart

Step 1.	Prepare reagents and samples
	↓
Step 2.	Dispense 100 µL/well of Dilution buffer, Standards and samples
	↓
	Incubate 30 minutes at 37°C
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 3.	Dispense 100 µL/well of Px-conjugate r.t.u.
	↓
	Incubate 30 minutes at 37°C
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 4.	Dispense 100 µL/well of TMB substrate
	↓
	Incubate 15 minutes in dark at room temperature
	↓
Step 5.	Dispense 100 µL/well of Stop solution
	↓
Step 6.	Read the absorbance at 450 / 620-690 nm within 10 min

General Information

Materials Supplied

Component	Amount
ELISA break-away strips coated with specific recombinant antigens	96 wells
Standard A = negative control serum, r.t.u.[1), 2)]	1.3 mL
Standard B, r.t.u.	1.3 mL
Standard C, r.t.u.	1.3 mL
Standard D = calibrator, r.t.u.	1.3 mL
Standard E = positive control serum, r.t.u.	1.3 mL
Anti-human IgM antibodies labeled with horseradish peroxidase (Px-conjugate) r.t.u.	13 mL
Wash buffer concentrate, 10x concentrated	55 mL
Dilution buffer anti-Borrelia rec., r.t.u.	60 mL
Chromogenic substrate (TMB substrate), r.t.u.	13 mL
Stop solution, r.t.u.	13 mL

1) r.t.u. = ready to use
2) The antibody concentration for each Standard (A-E) is mentioned in enclosed Quality control certificate AU/mL (Artificial units/mL).

Storage Instruction

- ✓ Store the kit and the kit reagents at 2 to 10°C, in a dry place and protected from the light. Store unused strips in the sealable pouch and keep the desiccant inside.
- ✓ Store undiluted serum samples at 2 to 10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.
- ✓ Do not store diluted samples. Always prepare fresh.
- ✓ If you find damage at any part of the kit, please inform the manufacturer immediately.
- ✓ Expiration date is indicated at the ELISA kit label and at all reagent labels.

Materials Required but Not Supplied

- ✓ Distilled or deionised water for diluting of the Wash buffer concentrate.
- ✓ Appropriate equipment for pipetting, liquid dispensing and washing.
- ✓ Thermostat (set at 37°C) for ELISA plate incubation.
- ✓ Spectrophotometer/colorimeter/microplate reader – wavelength 450 nm (and 620-690 nm reference filter - recommended, not required).

Precautions for Use

✓ Safety Precautions

- All ingredients of the kit are intended for laboratory use only.
- Standards contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.
- Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine.
- Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.
- The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin, …) in concentrations recommended by producer.
- Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.
- Do not smoke, eat or drink during work.
- Do not pipette by mouth.
- Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards.
- Avoid spilling or producing aerosol.

✓ Handling Precautions

- Manufacturer guarantees performance of the entire ELISA kit.
- Follow the assay procedure indicated in the Instruction manual.
- Controls (Standards), TMB substrate, Dilution buffer and Px-conjugate contain preservative ProClin 300®.
- Avoid microbial contamination of serum samples and kit reagents.
- Avoid cross-contamination of reagents.
- Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
- Variations in the test results are usually due to:
    * Insufficient mixing of reagents and samples
    * Inaccurate pipetting and inadequate incubation times
    * Poor washing technique or spilling the rim of well with sample or Px-conjugate
    * Use of identical pipette tip for different solutions

Assay Protocol

Reagent Preparation

- ✓ Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.
- ✓ Vortex samples (sera and cerebrospinal fluids) and the Standards in order to ensure homogeneity and mix all solutions well prior use.
- ✓ Dilute serum samples 1:100 (101x) in Dilution buffer and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). Dilute cerebrospinal fluid samples 1:1 in Dilution buffer (e.g. 75 µL of the cerebrospinal fluid sample + 75 µL of the Dilution buffer). Dilute synovial fluid samples 1:80 (81x) in Dilution Buffer (e.g. 5 µL of the synovial fluid sample + 400 µL of the Dilution buffer). Do not dilute Controls (Standards), they are ready to use.
- ✓ Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to 32 - 37°C in a water bath. Diluted Wash buffer is stable for one month if stored at room temperature.
- ✓ Do not dilute Px-conjugate, TMB substrate and Stop solution, they are ready to use.

Assay Procedure

1. Allow the microwell strips sealed inside the aluminium bag to reach room temperature. Withdraw an adequate number of strips and put the unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
2. Choose the method you intend to use (qualitative, semiquantitative or quantitative analysis, see below and/or Data Analysis section) and pipette Standards and samples according to the Plate Layout 1. Start with filling the first well with 100 µL of Dilution buffer to estimate the reaction background. In case of choosing the qualitative or semiquantitative method, fill two wells with 100 µL/well of Standard D (serves as calibrator), next well with Positive control serum (Standard E) and next one well with 100 µL of Standard A (negative control). In case of quantitative method, pipette all Standards as singlets (Standard A, B, C, D, E) (Plate Layout 2). Fill the remaining wells with 100 µL of diluted samples (S1, S2, S3,...). It is sufficient to apply samples as singlets, however, if you wish to minimize the laboratory error apply the samples in doublets. We recommend to include positive reference serum sample (your in-house internal control) into each run to follow the sequence, variability and accuracy of calibration.
3. Incubate 30 minutes (±2 min) at 37°C.
4. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µL/well of Wash buffer. Avoid cross-contamination between wells! If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.
5. Mix well the bottle with Px-conjugate and add 100 µL of Px-conjugate r.t.u. into each well.

6. Incubate 30 minutes (±2 min) at 37°C.
7. Aspirate and wash four tines with 250 µL/well of Wash buffer.
8. Dispense 100 µL of TMB substrate into each well.
9. Incubate 15 minutes (±30 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.
10. Stop the reaction by adding 100 µL of Stop solution (STOP). Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate few times to ensure complete mixing of the reagents.
11. Measure the absorbance at 450 nm with a microplate reader within 10 minutes. It is recommended to use a reference reading at 620-690 nm.

Data Analysis

Calculation of Results

Regardless of the method chosen, begin the processing with subtraction of the absorbance of the Dilution Buffer well (background absorbance) from the absorbances in all other wells.

✓ Processing of results for the Qualitative interpretation

1. Compute the mean of Calibrator (Standard D) absorbance from the two corresponding wells.
2. Compute the cut-off value by multiplying the Calibrator mean with the Correction factor. The Correction factor value for particular Lot is written in enclosed Quality control certificate.
3. Samples with absorbances lower than 90% of the cut-off value are considered negative and samples with absorbances higher than 110% of the cut-off value are considered positive.
4. For results of cerebrospinal fluid and synovial fluid see the next paragraph, Semiquantitative interpretation.

✓ Processing of results for the Semiquantitative interpretation

Determine the Positivity Index for each serum samples as follow:

1. Compute the cut-off value using the Calibrator (Standard D) mean and the Correction factor (see the previous paragraph)
2. Compute the Positivity Index for each sample according to the following formula:

$$\text{Sample Positivity Index} = \frac{\text{sample absorbance}}{\text{cut-off value}}$$

3. Express a sample reactivity according to Table 1 (Semiquantitative interpretation of results)

Table 1: Semiquantitative interpretation of results.

a. Blood Serum samples

Positive Index	Interpretation
< 0.90	Negative
0.90 - 1.10	+/-
>1.10	Positive*

b. Cerebrospinal fluid samples

Positive Index	Interpretation
< 1.00	Negative
1.00 - 1.40	+/-
>1.40	Positive*

c. Synovial fluid samples

Positive Index	Interpretation
< 1.00	Negative
1.00 - 1.30	+/-
>1.30	Positive*

**on the basis of the positivity index value it is possible to estimate semiquantitatively the amount of antibodies in the sample.*

Note: An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample.

Example of calculation:

Standard D absorbances	= 1.054; 1.089; 1.015
Standard D mean	= 1.053
Correction factor	= 0.14
Cut-off value	= 1.053*0.14 = 0.147
Sample absorbance	= 0.712
Sample Positivity Index	= 0.712/0.147 = 4.84

✓ Processing of results for Qualitative interpretation

Compute the sample antibody titre in artificial units (AU/ml) as follows:

1. Construct the calibration curve by plotting the absorbance of Standards (y-axis – in linear scale) to antibody concentration in artificial units (AU/mL) (x-axis – can have logarithmic scale). The antibody concentration in each Standard (A-E) is mentioned in enclosed Quality control certificate.
2. Determine the unknown antibody titres in samples from the calibration curve. It is possible to use a various software application for the standard curve fitting and for the calculation of the unknowns, e.g. Winliana, KimQ. For better fitting, the polynomic (four-parameter) function is most convenient.
3. Calibration curve and units of standard are related to serum diluted 101x. By other dilution of serum, the cerebrospinal fluid (e.g. 2x) or the synovial fluid (e.g. 81x) you obtained, using the calibration curve, the number of units in the sample (AU/sample). These units must be converted to the AU/mL according to this formula:

$$\frac{\text{AU/sample} * \text{dilution of sample}}{101} = \text{AU/mL}$$

Note! If the sample absorbance is out of the calibration curve (above the upper limit), repeat the test with more diluted (or less diluted) sample, for serum sample e.g. 1:200 or 1:400 (1:50) for serum sample, 1:10 or 1:20 for cerebrospinal fluid sample.

Result interpretation:

a. Blood serum samples

Concentration (AU/ml)	Interpretation
< 18.0	Negative
18.0 - 26.0	+/-
> 26.0	Positive

b. Cerebrospinal fluid samples

Concentration (AU/ml)	Interpretation
< 0.14	Negative
0.14 - 0.26	+/-
> 0.26	Positive

c. Synovial fluid samples

Concentration (AU/ml)	Interpretation
< 7.00	Negative
7.00 - 11.00	+/-
> 11.00	Positive

Note! An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample test. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample.

✓ Detection of Intrathecally produced IgM Antibodies

- Introduction

Detection of the intrathecal synthesis of IgM antibodies (detection of local synthesis of specific IgM antibodies in the central nervous system) is necessary for the diagnosis of the early and the late neuroborreliosis. It requires measurement of specific IgM antibodies in blood serum and in cerebrospinal fluid and the determination of albumin and the total IgM level in both samples. The intrathecal synthesis of antibodies is determined as specific antibody index (AI) and calculated from the antibody concentration ratio in cerebrospinal fluid and blood serum in relation to the status of blood-cerebrospinal fluid barrier.
Note: If both serum and cerebrospinal fluid samples give negative results, do not count antibody index AI (intrathecal synthesis of specific antibodies is not expected).

- Calculation of the antibody index (AI)

1. Calculate the ratio of the total IgM concentration in cerebrospinal fluid to the total IgM concentration in serum ($Q_{total\ IgM}$) and the ratio of the cerebrospinal albumin level to the serum albumin level ($Q_{total\ alb}$).

$$Q_{total\,IgM} = \frac{total\,IgM\ in\ CSF}{total\,IgM\ in\ blood\ serum} \qquad Q_{total\,alb} = \frac{albumin\ in\ CSF}{albumin\ in\ serum}$$

Example of calculation:

total IgM in CSF = 0.065 g/L

total IgM in serum = 17.29 g/L

albumin in CSF = 0.272 g/L

albumin in serum = 30.64 g/L

$$Q_{\text{total IgM}} = \frac{0.065}{17.29} = 3.76*10^{-3}$$

$$Q_{\text{total alb}} = \frac{0.272}{30.64} = 8.88*10^{-3}$$

2. Calculate the limiting quotient $Q_{\text{lim IgM}}$ which is the amount of IgM found in cerebrospinal fluid that can originate from the systemic circulation (hyperbolic function according to Reiber et al. Clin Chem 37/7, 1153-1160 (1991)).

Compute Q_{lim} using the equation:

$$Q_{\text{lim IgM}} = 0.93*\sqrt{(Q_{\text{total alb}})^2 + 6*10^{-6}} - 1.7*10^{-3}$$

Example of calculation:

$$Q_{\text{lim IgM}} = 0.93*\sqrt{(8.88*10^{-3})^2 + 6*10^{-6}} - 1.7*10^{-3}$$

$$Q_{\text{lim IgM}} = 6.86*10^{-3}$$

3. Calculate the ratio of concentration of specific IgM antibodies in CSF to concentration of specific IgM in serum $Q_{\text{spec IgM}}$.

$$Q_{\text{spec IgM}} = \frac{\text{spec.IgM CSF} * \text{sample dilution}}{\text{spec.IgM serum} * \text{sample dilution}}$$

Where spec. IgM CSF is the concentration of specific antibodies in AU/mL in cerebrospinal fluid and spec. IgM serum is the concentration of specific antibodies in AU/mL in serum.

Example of calculation:

spec. IgM CSF = 38 AU/mL, sample diluted 2 times in dilution buffer

spec. IgM serum = 10 AU/mL, sample diluted 101 times in dilution buffer

$$Q_{\text{spec IgM}} = \frac{38*2}{10*101} = 75.2*10^{-3}$$

4. Calculation of antibody index AI

a. If $Q_{\text{total IgM}} < Q_{\text{lim IgM}}$, then calculate AI using the formula:

$$AI = \frac{Q_{\text{spec.IgM}}}{Q_{\text{total IgM}}}$$

Example of calculation:

$Q_{total\ IgM} = 3.76 * 10^{-3}$

$Q_{lim\ IgM} = 6.68 * 10^{-3}$

$Q_{spec.IgM} = 75.2 * 10^{-3}$

$Q_{total\ IgM} = 3.76 * 10^{-3} < Q_{lim\ IgM} = 6.68 * 10^{-3}$

AI = 0.0752/0.00376 = 20

b. If $Q_{total\ IgM} > Q_{lim\ IgM}$, compute AI using the formula:

$$AI = \frac{Q_{spec.IgM}}{Q_{lim\ IgM}}$$

Example of calculation:

$Q_{total\ IgM} = 13.5 * 10^{-3}$

$Q_{lim\ IgM} = 6.86 * 10^{-3}$

$Q_{spec.IgM} = 75.2 \times 10^{-3}$

$$AI = \frac{0.0752}{0.00686} = 11$$

- Result interpretation (according to Reiber)

AI value	Interpretation
< 1.3	negative, intrathecal synthesis not proven
1.3 - 1.5	equivocal
> 1.5	positive, intrathecal synthesis proven

Note: If the sample absorbance is out of the calibration curve (above the upper limit), repeat the test with different sample dilution.

Performance Characteristics

✓ Validity of the test

The test is valid if:

The background absorbance (the absorbance of the Dilution buffer) is less than 0.150. The mean absorbance values of standards/control sera, and the ratio between the absorbance values of Standard E/Standard D are in the range stated in the Quality control certificate for the kit lot. The test is intended for the detection of IgM antibodies in human serum, cerebrospinal fluid, synovial fluid.

✓ Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples of different absorbance values.

- Intraassay variability

  The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot at least on 12 parallels of the same microtitration plate.

  Example: N = number of parallels of the same microtitration plate, SD = standard deviation):

N	Mean absorbance	SD	CV%
16	1.335	0.050	3.8%
16	0.614	0.023	3.7%

- Interassay variability

  The coefficient of intraassay variability is max. 15%. It is measured for each particular Lot as comparison of the OD values of the same serum sample in several consecutive tests.

  Example: (N = number of an independent examination of the same serum sample, SD = standard deviation):

N	Mean Absorbance	SD	Range (min.-max.)	CV%
18	1.369	0.064	1.223-1.476	4.7%
43	1.372	0.119	1.184-1.750	8.7%

- Recovery test

  Measured values of recovery test for every Lot are between 80-120% of expected values.

✓ Sensitivity and specificity

The sensitivity of the test is 95% and the specificity is 99%. Evaluation was performed with a set of blood samples that comprise of : a) anti-borrelia IgM positive sera, b) anti-borrelia IgM negative sera. Results were confirmed by other commercially available test during the internal validation testing and external validation testing.

✓ Limitation of quantification

The limit of quantification is 2.41 AU/mL. The limit of quantification was defined as the lowest measurable concentration which can be distinguished with 95% confidence from zero.

✓ Interference

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 80 mg/mL of triglycerides.

Resources

Plate Layout 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dilution Buffer	Sample 4										
B	Standard D (Calibrator)											
C	Standard D (Calibrator)											
D	Standard E (Positive control)											
E	Standard A (Negative control)											
F	Sample 1											
G	Sample 2											
H	Sample 3											

Qualitative and Semiquantitative analysis

Plate Layout 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dilution Buffer	Sample 3										
B	Standard A (Negative control)											
C	Standard B											
D	Standard C											
E	Standard D (Calibrator)											
F	Standard E (Positive control)											
G	Sample 1											
H	Sample 2											

Quantitative analysis