LIA-VIDITEST anti-*Borrelia* IgM

ODZ - 317

Instruction manual

PRODUCER:
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1. TITLE:

LIA-VIDITEST anti-Borrelia IgM – Line ImmunoAssay with high diagnostic sensitivity and specificity - confirmatory assay.

2. INTENDED USE:

LIA-VIDITEST test kit is intended for qualitative detection of specific IgM antibodies against antigens of major pathogenic Borrelia stains (***B. afzelii, B. garinii a B. burgdorferi sensu stricto***) and ***Anaplasma phagocytophila*** (HGA) in human serum and plasma. The assay is used for the confirmation of ELISA results for serological diagnosis of Lyme disease (LD). The diagnosis of LD is based on the combination of clinical examination and laboratory testing. Anti-borrelia IgM antibodies are detectable 2-3 weeks after infection, they reach maximum 6 weeks after infection, then their levels generally decrease, followed by IgG antibody. Determination of specific antibodies in the early stage of infection is particularly important in cases where no obvious typical clinical symptoms (e.g. erythema migrans - only 50% of cases).

However, clinical symptoms of LD are similar to other diseases, therefore the serological methods are of use in differential diagnosis of neuroinfections, arthropathies, carditis and skin diseases.

When interpreting the results should take into account the slow formation of antibodies in the early stage of the disease, the possibility of influencing antibody production of antibiotics, low percentage of seronegative patients, the possibility of cross reactions in patients with other diseases. Therefore, it is always necessary to compare the results with clinical data (taken from the NRL for LD).

The kit can be also used to an indicative diagnostics of Human granulocytic anaplasmosis (HGA). HGA is caused by bacterium ***Anaplasma phagocytophila***, which attack white blood cells (granulocytes). Anaplasma infection can result in death for immunodefinicient, post-splenectomy or post-transplant patients. Anaplasma specific antibodies are detected in human serum/plasma by high-specific antigen p44.

3. TEST PRICNIPLE:

LIA-VIDITEST anti-*Borrelia* is confirmatory test for qualitative detection of specific IgM antibodies in human serum/plasma. The test is based on high-specific recombinant antigens immobilized to nitrocellulose membrane strips. These immobilized antigens react with specific antibodies from serum/plasma and captured antibodies are detected in next step by animal anti-human IgM antibodies labelled with alkaline phosphatase. The presence of labelled antibodies is revealed by an enzymatic reaction with a chromogenic substrate. In the presence of the antigen specific antibodies color band will appear on relevant lines on membrane and color intensity is directly proportional to the antibodies concentration in sample.

4. KIT COMPONENTS:

Nitrocellulose membrane strips coated with specific recombinant antigen lines STRIPS	2 x 8 strips
15 mL Anti-human IgM antibodies labeled with alkaline phosphatase r.t.u. CONJ	2 vials
125 mL Universal buffer r.t.u.* BUF UNI	2 vials
15 mL Substrate for AP (NBT/BCIP) r.t.u. SUBS	2 vials
Instruction manual	1 piece
Evaluation protocol	2 pieces
Adhesive film	2 pieces
Quality control certificate	1 piece

*r.t.u., ready to use

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

Distilled or deionised water, appropriate 1 and 8- channel pipetting equipment, forceps, shaker.

6. ASSAY PROCEDURE:

a. Allow all kit components to reach room temperature.

b. After that strips reach room temperature, withdraw an adequate number of strips from packaging. Insert strips into incubation tray in increasing number order by identification code on each strip, one strip per well, code-side upside. Using forceps hold the end of the strip with the identification code and take special care for all manipulation with strips. Unused strips put back into the packaging.

c. Blocking of strips: Mix the universal buffer in vial carefully. Add 1.5 mL of universal buffer into each well of incubation tray. Ensure that all strips are submerged completely. If necessary, remove bubbles using a pipette tip. Incubate with gentle shaking (app. 160 RPM) for **15 min** (± 1 min) at room temperature.

d. Prepare samples to testing during blocking of strips. All samples should be stirred well before processing. Each sample dilutes 101x in universal buffer. (E.g. for one well mix 17 µl of serum/plasma with 1.7 mL of universal buffer).

e. Precisely aspirate the liquid from incubation tray wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions) using 8-chanell pipette or other appropriate pipetting equipment). Add 1.5 mL of the diluted samples into each well of incubation tray and ensure that all strips are submerged completely. If necessary, remove bubbles using a pipette tip. Incubate with gentle shaking for **30 min** (± 2 min) at room temperature.

f. Aspirate the liquid from the incubation tray wells (see step e.). Wash each strip three times by 1.5 mL of universal buffer. Incubate strips in all washing steps with gentle shaking for 5 min (± 5 s) at room temperature and aspirate the solution precisely.

g. Add 1.5 mL of the anti-IgM AP conjugate working solution to all incubation tray wells and ensure that strips are submerged completely. Incubate with gentle shaking for **30 min** (± 2 min) at room temperature.

h. Wash the strips same way as in step f.

i. Add 1.5 mL of substrate for AP to all incubation tray wells and ensure that strips are submerged completely. Cover the strips with an aluminum foil or keep them in the dark during incubation. Incubate with gentle shaking for **10 min** (± 10 s) at room temperature.

j. Aspirate all substrate from the incubation tray wells and wash all strips twice with 1.5 mL of distilled/deionised water. Incubate strips in all washing steps with gentle shaking for **1 min** (± 5 s) at room temperature and aspirate the water precisely.

k. Use forceps to transfer strips from incubation tray and put them on filtration paper or gauze. Let the strips to dry at room temperature.

Note! LIA-VIDITEST anti-*Borrelia* IgG and IgM tests can be performed in parallel when using autoautomatic or semiautomatic analyzers. Universal buffer and Substrate for AP are interchangeable between LIA-VIDITEST anti-*Borrelia* IgM and IgG. Assay procedure and incubation times are identical for both kits (IgG and IgM). Test is validated for the Dynablot analyzer.

7. PROCESSING OF RESULTS:

7.1. Description of the of membrane strips

The strip contains 3 control lines and 9 antigen lines, as listed in table 1.

Tab. 1: List of antigens lines on the membrane strip.

IgM conjugate control	positive control
IgG conjugate control	negative control
cut-off control	give an interface between negativity and positivity
OspC (p25)	mixture of antigens OspC *B. afzelii*, *garinii* and *burgdorferi sensu stricto*, major early antigen, highly specific
FlaB (p41)	mixture of antigens FlaB *B. afzelii*, *garinii* and *burgdorferi sensu stricto*, often nonspecific
DbpA (Osp17)	mixture of antigens DbpA *B. afzelii*, *garinii* and *burgdorferi sensu stricto*, early and late antigen, highly specific
BmpA (p39)	mixture of antigens BmpA *B. afzelii*, *garinii* and *burgdorferi sensu stricto*, early and late antigen, highly specific
OspA-LT *B. afzelii* (p31)	early antigen, low sensitive, species-specific
OspA-LT *B. garinii* (p31)	early antigen, low sensitive, species-specific
OspC-GV *B. afzelii* (p25)	early antigen, species-specific
OspC-GV *B. garinii* (p25)	early antigen, species-specific
p44 *Anaplasma phagocytophila*	highly specific antigen *Anaplasma phagocytophila*

7.2 Evaluation of results

Manual evaluation:

1) To prepare evaluation protocol remove covering paper from sticky tape in frame dedicated for strips. Hold the dried strips by labeled end and very carefully stick to the frame on the evaluation protocol. Ensure that positive control line on strip is placed in alignment to the positive control line on printed template.

2) Check that the test was carried out correctly and is valid according to the criteria of the test.

The validity of the test:

The test is valid if:

a) on all strips are intensive lines of IgM conjugate control and no or very weak lines of IgG conjugate control.
b) on all strips are present lines of cut-off control.

In other case, the test cannot be evaluated and should be repeated.

3) Write in the boxes relevant antigens presence or absence of visible lines under cut-off control as follows:

Specific lines with intensity higher as intensity of cut-off are positive, mark by "+".

Specific lines with intensity lower as intensity of cut-off are negative, mark by "neg" or leave the box blank.

Intensity of the specific lines similar to intensity of cut-off are around threshold, mark by "±".

Note: Even negative serum/plasma can induce a weak signal that is only the background of reaction.

4) Assign points to all lines according the table 2. Count points for each sample and write result into "Result" box. According to table 3 evaluate the test samples.

5) Finally cover the strips area with an adhesive foil.

Tab. 2: Assign of points

Borrelia antigens (antigen p44 is evaluated separately)	**line**		
	strong +	weak +/-	none -
OspC or OspC-GV *B. afzelii* or OspC-GV *B. garinii*	20	3	0
FlaB	2	1	0
DbpA, BmpA, OspA-LT *B. afzelii* or *B. garini*	5	1	0

Note:
Species-specific antigens are used for more accurate detection of antibodies with species distinctions. I.e. if there are present the lines for DbpA, DbpA-TT B. afzelii or DbpA-TT B. garinii, do not add points for all these lines, but in the total sum of points is included only 1 line with the highest number of points.

Tab.3: Evaluation of results

points	**Interpretation**
0 - 2	negative
3 - 7	borderline
8 - 37	positive

Evaluation of p44 antigen line (*Anaplasma phagocytophila*)
According the cut-off control evaluate the p44 line as described in chapter 7.2, section 3. In case both antibodies against Borrelia and p44 antigens simultaneously occur, may be a combined infection caused by bacteria *Borrelia sp.* and even *Anaplasma phagocytophila*.
It is necessary to consider the possibility of cross-reactions of the anti-Borrelia antibodies with antigens of other spirochetes.

Software evaluation:

1) Follow the step described in item 1) of manual evaluation.
Note: Make sure that the strips are thoroughly dried before sticking them in the evaluation protocol frame. Sticking the wet or moist strips on the evaluation protocol may cause the paper rippling and consequently an error when scanning the evaluation protocol. Do not write any notes to around the frame dedicated for strips. This may cause an error during loading the software field evaluation protocol.

2) Use software VidiaScan to scan evaluation protocol and evaluate the results according to the software manual.

7.3. Interpretation of the results:

Diagnosis (stage)	**Clinical symptoms**	**Laboratory test**	
		basic	supporting
I. The early localized infection	Erythema migrans	IgM positive (3-6 weeks post-infection) **often seronegative**	skin biopsy
II. early disseminated infection	Borrelia-lymfocytom Myocarditis, Oftalmoborreliosis Neuroborreliosis	**IgM positive, IgG positive**, or IgM neg., IgG pos.	Histological evidence of B-cell pseudolymphocytoma
III. late disseminated infection	Arthritis, Acrodermatitis chronica atrophicans, Chronic neuroborreliosis	**IgM neg., IgG pos**. (high titers of IgG antibodies)	

8. CHARACTERISTICS OF THE TEST:

8.1. Diagnostic sensitivity and specificity

The diagnostic sensitivity of the test is 97% and the specificity is 98%. Evaluation was performed with a set of blood samples that comprised of: a) anti-Borrelia IgM positive sera, b) anti-Borrelia IgM negative sera. Results were confirmed by other commercially available diagnostic test during the internal validation testing.

8.2 Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples with different concentrations of specific antibodies. The samples were according to the color intensity of antigenic lines.

8.2.1. Intraassay variability

The coefficient of intraassay variability (v%) is max. 5%. It is measured for each particular Lot at least on 4 parallels of membrane strips.

Example: (N = number of parallels of membrane strips, SD = standard deviation)

		Color intensity - serum 9233583 (positive control)								
strip code	gray zone	OspC	FlaB	DbpA	BmpA	OspA-LT afzelii	OspA-LT garinii	OspC-GV afzelii	OspC-GV garinii	p44
007M31	230-235	217	207	244	227	159	232	242	239	208
007M32	232-237	214	203	244	226	169	233	244	243	211
007M33	232-237	214	205	245	227	163	236	247	244	208
007M34	233-238	219	209	246	229	169	236	247	244	210
mean		216	206	245	227	165	234	245	243	209
SD		2,449	2,582	0,957	1,258	4,899	2,062	2,449	2,380	1,500
v%		**1%**	**1%**	**0%**	**1%**	**3%**	**1%**	**1%**	**1%**	**1%**
min		214	203	244	226	159	232	242	239	208
max		219	209	246	229	169	236	247	244	211
N		4	4	4	4	4	4	4	4	4

Note: The positive value of the signal (intensity antigenic color lines) are shown in red, blue borderline and black negative.

8.2.2. Interassay variability

The coefficient of interassay variability (v%) is max. 15%. It is measured for each particular Lot as comparison of antigen lines color intensity of the same serum/plasma sample in several consecutive tests.

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

		Color intensity - serum 9233583 (positive control)								
strip code	gray zone	OspC	FlaB	DbpA	BmpA	OspA-LT afzelii	OspA-LT garinii	OspC-GV afzelii	OspC-GV garinii	p44
008M05	224-229	201	187	236	230	144	223	239	237	200
008M20	229-234	199	188	239	232	155	227	237	235	202
007M05	224-229	198	185	240	211	134	224	235	235	194
007M20	228-233	210	199	240	223	170	230	240	239	206
009M01	229-234	203	184	239	238	136	227	237	237	208
002M35	218-223	197	183	236	222	157	224	232	234	153
mean	225-230	201	188	238	226	149	226	237	236	194
SD		4,761	5,854	1,862	9,445	13,852	2,639	2,875	1,835	20,595
v%		**2%**	**3%**	**1%**	**4%**	**9%**	**1%**	**1%**	**1%**	**11%**
min		197	183	236	211	134	223	232	234	153
max		210	199	240	238	170	230	240	239	208
N		6	6	6	6	6	6	6	6	6

8.3. 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.

9. SAFETY PRECAUTIONS:

All ingredients of the kit are intended for laboratory use only.

The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin) in concentrations recommended by the producer.

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.

10. HANDLING PRECAUTIONS:

a. Manufacturer guarantees performance of the entire LIA kit. Avoid mixing different lots of reagents.
b. Avoid microbial contamination of serum/plasma samples and kit reagents.
c. Avoid cross-contamination of reagents.
d. Universal buffer contain preservative ProClin 300®.
e. Avoid contact of the substrate for AP with oxidizing agents or metal surfaces.
f. Follow the assay procedure indicated in the Instruction manual. Variations in the test results are usually due to:

- Insufficient mixing of reagents and samples.
- Insufficient submerging of strips in reagents or presense of bubbles.
- Inaccurate pipetting and inadequate incubation times.
- Poor washing technique or insufficient aspiration of liquid from incubation tray.
- Using the same tip when pipetting different solutions or substitution of caps.

11. STORAGE AND EXPIRATION:

Store the kit reagents at +2 to +10°C, in a dry place and protected from the light. Expiration date is indicated at the LIA kit label and at all reagent labels.

Kits are shipped in cooling bags. If you find damage at any part of the kit, please inform the manufacturer immediately.

Unused strips put with the desiccant back into the packaging.

Do not store diluted samples. Always prepare fresh.

For long-term storage keep the unused serum/plasma samples in undiluted state and frozen in aliquots at -18 to -28°C. Avoid repeated thawing and freezing. Store serum/plasma samples at +2 to +10°C up to 3 days.

12. FLOW CHART:

Step 1.	Prepare reagents and samples; allow all components to reach room temperature.
	↓
Step 2.	Blocking of strips: Add 1.5 mL/well of universal buffer to each strip. ↓ Incubate **15 min** at room temperature with shaking. ↓ Aspirate the well contents.
	↓
Step 3.	Add 1.5 mL of 101x diluted serum/plasma sample to each strip. ↓ Incubate **30 min** at room temperature with shaking. ↓ Wash strips 3 times for 5 min by 1.5 mL of universal buffer.
Step 4.	Add 1.5 mL of anti-IgM AP conjugate r.t.u. to each strip. ↓ Incubate **30 min** at room temperature with shaking. ↓ Wash strips 3 times for 5 min by 1.5 mL of universal buffer.
	↓
Step 5.	Add 1.5 mL of AP substrate to each strip. ↓ Incubate in dark **10 min** at room temperature with shaking.
	↓
Step 6.	Aspirate the well contents and wash strips 2 times for 1 min by 1,5 mL of distilled/deionised water at room temperature with shaking.
	↓
Step 7.	Dry and stick strips on evaluation protocol.

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