LIA-VIDITEST
anti-Borrelia IgM garinii

REF ODZ-493 16 tests 2°- 10 °C

Type of determination: IgM antibodies

Type of evaluation: Qualitative

Type of samples: Serum/Plasma/Cerebrospinal fluid/Synovial fluid

Processing: Manual and/or semi-automatic/automatic

RoboBlot™, BeeBlot™, B20

VIDIA

CE IVD

Instruction manual

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1. TITLE

LIA-VIDITEST anti-Borrelia IgM garinii

2. INTENDED USE

The kit is intended for professional use for qualitative detection of specific IgM antibodies against antigens of Borrelia (***B. garinii)*** in human serum, plasma, cerebrospinal fluid and synovial fluid. This confirmatory test is used for the confirmation of ELISA results in serological diagnostics of Lyme disease (LD). The diagnostics of LD is based on the combination of clinical examination and laboratory testing. Anti-borrelia IgM antibodies are produced 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 can be similar to other diseases, therefore the serological methods are of use in differential diagnostics of neuroinfections, arthropathies, carditis, and skin diseases.

3. TEST PRINCIPLE

LIA-VIDITEST anti-Borrelia is confirmatory test for qualitative detection of specific IgM antibodies in tested samples. The test is based on high-specific recombinant antigens immobilized onto nitrocellulose membrane strips. The principle of the test is that these immobilized antigens react with specific IgM antibodies. The specific antibodies are indirectly detected in the next step using secondary antibody labelled with alkaline phosphatase. The amount of the bound labelled antibodies is detected after the addition of alkaline phosphatase by reaction with a chromogenic substrate. In the presence of the antigen specific antibodies colour band will appear on relevant lines.

4. KIT COMPONENTS

nitrocellulose membrane strips coated with specific recombinant antigen lines STRIPS	16 strips
20 mL Anti-human IgM animal antibodies labelled with alkaline phosphatase (conjugate anti-IgM AP) r.t.u.[1] CONJ-L	2 vials
120 mL Universal buffer r.t.u. BUF UNI	2 vials
20 mL Substrate for AP (NBT/BCIP) r.t.u. SUBS	2 vials
Instruction manual	1 piece
Evaluation protocol	3 pieces
Adhesive foil	3 pieces
Quality control certificate	1 piece

[1] r.t.u., ready to use

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Distilled or deionized water, incubation trays, pipettes (1-channel, 8-channel pipettes manual or automatic), forceps, and shaker.
The test can be processed semi-automatically using Dynablot device or automatically using RoboBlot™, BeeBlot™ and B20.
Functional validation for all the machines and devices used must be valid.

6. PREPARATION OF REAGENTS

a. Allow all kit components to reach room temperature.
b. Mix universal buffer BUF UNI, conjugate anti-IgM AP CONJ-L and substrate for AP SUBS thoroughly
c. Mix tested samples well prior use.

7. ASSAY PROCEDURE

The manufacturer is not responsible for the correct function of the kit if the assay procedure is not followed.

a. 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.
b. Blocking of strips: Mix well the vial with universal buffer. **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 (it is recommended using clean pipette tip). Incubate with gentle shaking (160 RPM) for **15 min** (± 1 min) at room temperature.
c. Prepare serum/plasma, cerebrospinal fluid and synovial fluid samples to be tested during strips blocking. After thorough mixing of the samples and universal buffer dilute them as following:

   serum/plasma dilute 101x in universal buffer.

   E.g. for one well: mix **17 µL of serum/plasma + 1.7 mL of universal buffer**

   cerebrospinal fluid samples dilute 2x in universal buffer.

   E.g. for one well mix: **750 µL of cerebrospinal fluid + 0,750 mL of universal buffer**

   synovial fluid samples dilute 81x in universal buffer.

   E.g. for one well mix: **20 µL of cerebrospinal fluid + 1, 6 mL of universal buffer**

   NOTICE:

   If the amount of cerebrospinal fluid sample is limited, it is possible to use a smaller amount of diluted cerebrospinal fluid (2x in universal buffer), e.g. 500 µL of cerebrospinal fluid + 500 µL of universal buffer, but at least 350 µL cerebrospinal fluid + 350 µL universal buffer. Make sure that the entire surface of the strip is immersed in the cerebrospinal fluid sample!

   Hemolytic serum testing is not recommended!
d. Precisely aspirate the liquid from incubation tray wells into a waste bottle containing an appropriate disinfectant (see paragraph 11. WARNINGS) using 8-chanell pipette or other appropriate pipetting equipment. **Add 1.5 mL of the diluted samples into each well of incubation tray**.

   It is recommended to include into each run a sample of positive reference serum (internal control) for verification of continuity and variability of the test. Ensure that all strips are submerged completely, if necessary, remove bubbles. Incubate with gentle shaking for **30 min** (± 2 min) at room temperature.
e. Aspirate the liquid from the incubation tray wells into a waste bottle containing an appropriate disinfectant (see paragraph 11. WARNINGS) using 8-chanell pipette or other appropriate pipetting equipment. Wash each strip **three times with 1.5 mL of universal buffer**, incubate strips in all washing steps with gentle shaking for **5 min** (± 5 s) at room temperature. Then aspirate the solution.
f. **Add 1.5 mL of the anti-IgM AP conjugate r.t.u. into all incubation tray wells.** Ensure that the strips are submerged completely, remove bubbles if necessary. Incubate with gentle shaking for **30 min** (± 2 min) at room temperature.
g. Wash the strips the same way as in step e.
h. **Add 1.5 mL of Substrate for AP to all incubation tray wells**. Ensure that strips are submerged completely, remove bubbles if necessary. Cover the strips with an aluminum foil or non-transparent lid.

Incubate with gentle shaking for **10 min** (± 5 s) at room temperature. Because the substrate is sensitive to light, close the vial immediately after use. The substrate must be clear during use. Do not use the solution, if it is blue coloured.

i. Aspirate all substrate from the incubation tray wells and **wash all strips twice with 1.5 mL of distilled/deionized water**, incubate strips in all washing steps with gentle shaking for **1 min** (± 5 s) at room temperature. Then aspirate the solution.

j. Use forceps to transfer strips from incubation tray and put them on filtration paper or gauze in order to avoid strips warping. Let the strips to dry at room temperature (a minimum of 1,5 hours).

Note. LIA-VIDITEST tests can be performed in parallel when using automatic or semiautomatic analysers. Assay procedure and incubation times are identical for all LIA-VIDITEST kits. Universal buffer and Substrate for AP are interchangeable between LIA-VIDITEST kits. The test is validated for Dynablot, B20, BeeBlot and RoboBlot analysers.

8. PROCESSING OF RESULTS

8.1. Description of the of membrane strips

The strip contains 3 control lines and 6 antigen lines, as listed in table 1.

Tab. 1

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

8.2 Evaluation of results

The results can be evaluated manually or using software. It is recommended software evaluation – it is more objective and more precise evaluation.

Check if 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) there is an intensive line of IgM conjugate control and non or very weak line of IgG conjugate control

b) cut-off control is presented on all strips

In other cases, the results cannot be evaluated and the test has to be repeated.

Note: In case the strip is inversely coloured (i.e. the dark background with white lines), evaluate this sample as negative.

Waning:

It is recommended to use the same lot of the strips within one Evaluation protocol.

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, beginning from the left side of the frame of the Evaluation protocol. Write down the sample name beside each strip into the prepared fields ("Sample").

2) Into the protocol, write down relevant antigen presence or absence of visible lines according to cut-off control as follows:

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

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

Intensity of the specific lines similar to intensity of cut-off evaluate as equivocal, mark by "±".

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

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

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

Tab. 2: Assigning of points

Borrelia antigens	**line**		
	strong +	**weak +/-**	**none -**
OspC *B. garinii*, OspC-GV *B. garinii*	**20**	**3**	**0**
FlaB *B. garinii*	**2**	**1**	**0**
DbpA *B. garinii*, BmpA *B. garinii*, OspA-LT *B. garinii*	**5**	**1**	**0**

Tab.3: Evaluation of results

points	**Interpretation**
0 - 2	negative
3 - 7	equivocal
≥ 8	positive

Software evaluation:

WARNING

In case of software evaluation and scanning of Evaluation protocol, the strip must not be covered by adhesive foil.

Note: Be sure that before sticking the strips onto the frame of the Evaluation protocols, the strips are carefully dried. Sticking wet or moist strips on the Evaluation protocol can cause warping of the paper and therefore the producing of mistake during the scanning of Evaluation protocol. Do not write any notes to the surrounding of the frame for strips sticking in order not to cause a failure of software reading of the Evaluation protocol.

1) Perform the step described in paragraph 1) of Manual evaluation
2) Scan the Evaluation protocol using VidiScan software and evaluate the results according to the software manual. If you are interested in the software, please, contact the manufacturer.

9. RECOMMENDED INTERPRETATION OF THE RESULTS

For results interpretation is important on to consider the slow antibodies production in early phase of the disease, the possibility of influence of previous antibiotics treatment, seronegativity at low percentage of infected persons, possibility of cross-reactivities in persons with other diseases. Therefore, it is necessary to compare the results with clinical data (reference from the National reference laboratory for Lyme disease, Czech Republic).

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

10. CHARACTERISTICS OF THE TEST

The kit is intended for professional use for qualitative detection of specific IgM antibodies against antigens of Borrelia (*B. gariniii)* in human serum (heparinized), plasma, cerebrospinal fluid and synovial fluid taken by standard laboratory procedure.

10.1. Diagnostic sensitivity and specificity

The determination of diagnostic sensitivity was performed by testing of patients' samples (n = 27), in which the positive result for IgM against borrelia was expected. The result was confirmed by independent commercial tests within internal diagnostic efficiency evaluation study. Diagnostic sensitivity is 97%.

Specificity of the test was determined by testing of characterized serum samples (n = 27), in which the absence of IgM against borrelia was expected. The result was confirmed by independent commercial tests within internal diagnostic efficiency evaluation study. Diagnostic specificity is 98%.

10.2 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 evaluated according to the colour intensity of antigenic lines.

10.2.1. Repeatability (intraassay)

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

Example:

(n = number of parallels of membrane strips in one incubation tray)

n	A	±σ	CV intra%
4	192	6.481	3 %
4	199	2.217	1 %
4	190	4.123	2 %

10.2.2. Reproducibility (interassay)

The coefficient of interassay variability (CV inter. %) is maximum 15%. It is measured for each particular Lot as comparison of antigen lines colour intensity of the same sample in several consecutive tests.

Example:

(n = number of an independent examination of the same sample)

n	A	±σ	min – max	CV inter
4	141	3.862	136-145	3 %
4	201	2.380	170-178	4 %
4	192	2.646	188-194	1 %

10.3. 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.

11. WARNINGS

a. All kit components are for laboratory use only.
b. The manufacturer guarantees the usability of the kit as a whole.
c. Work aseptically to avoid microbial contamination of samples and reagents.
d. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.
e. The substrate for AP should not come into contact with oxidizing agents and metal surfaces.
f. Follow the Instruction manual exactly. Non-reproducible results may arise in particular:
    - insufficient mixing of reagents and samples before use
    - insufficient immersing of strips or bubbles production
    - inaccurate pipetting and non-compliance with the incubation times given in Chapter 7
    - poor washing technique (e.g. insufficient aspiration of reagents from the wells)
    - using the same tips when pipetting the different solutions or swapping caps
g. Disinfect the waste generated during strips washing in a waste container using a suitable disinfectant solution (e.g. Incidur, Incidin, chloramine, ...) at the concentration recommended by the manufacturer.
h. Treat test specimens as infectious material. Autoclave items that have been in contact with them for 1 hour at 121 °C or disinfect for at least 30 minutes with 3% chloramine solution. All reagents and packaging material must be disposed of in accordance with applicable legislation.
i. Do not eat, drink or smoke while working. Do not pipette by mouth, but by suitable pipetting devices. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.
j. In case of suspicion of an adverse event in connection with the use of the kit, inform the manufacturer and the competent state authority without delay.

12. SAFETY PRECAUTIONS

Universal buffer BUF UNI is preserved with ProClin 300 (a mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)). Therefore, the following warnings and safety precautions apply to these solutions:

Warning

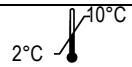
H317	May cause an allergic skin reaction.
P280	Wear protective gloves/protective clothing/ protective glasses/ face protection.
P302+P352	OF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

Further information can be found in the safety data sheet.

13. STORAGE AND EXPIRATION

a. Store the kit and the kit reagents at +2 °C to +10 °C, in a dry place and protected from the light. Under these conditions, the expiration period of the entire kit is indicated on the central label on the kit package, the expiration date of the individual components is indicated on their package.
b. The kits are transported refrigerated in thermal bags, If, upon receipt of the kit, serious damage to the packaging of any component of the kit is noticed, inform the manufacturer immediately.
c. Store unused serum/plasma, cerebrospinal fluid, synovial fluid samples undiluted, aliquoted and frozen at -18 °C to -28 °C. Frequent freezing and thawing is not recommended.
d. Diluted serum/plasma, cerebrospinal fluid, synovial fluid sample solutions at the working concentration cannot be stored. Always prepare them fresh.

14. USED SYMBOLS

Symbol	explanation
	number of tests
CE	Conformité Européenne – product meets the requirements of European legislation
IVD	diagnostics *in vitro*
±σ	standard deviation
CV	coefficient of variation
OD	optical density
	manufacturer
	expiration
LOT	lot of kit
	storage at +2 °C - +10 °C
°C	Celsius degree
%	percentage
n	number of tested samples
A	value of a certain sample
	read the package leaflet
REF	catalog number

15. FLOW CHART

Step 1.	**Prepare reagents and samples; allow all components to reach room temperature**

↓

Step 2.	**Blocking of strips: Add of universal buffer to each strip (1.5 mL/well)** ↓ **Incubate 15 min at room temperature with shaking** ↓ **Aspirate the well contents**

↓

Step 3.	**Add diluted serum/plasma/cerebrospinal fluid/synovial fluid sample (1.5 mL or lower volume in case of cerebrospinal fluids – see chapter 7, point c.)** ↓ **Incubate 30 min at room temperature with shaking** ↓ **Wash 3 times for 5 min (1.5 mL/well), aspirate**

Step 4.	**Add of anti-IgM AP conjugate r. t. u. (1.5 mL/well)** ↓ **Incubate 30 min at room temperature with shaking.** ↓ **Wash 3 times for 5 min (1.5 mL/well), aspirate**

↓

Step 5.	**Add 1.5 mL of AP substrate (1.5 mL/well)** ↓ **Incubate in dark 10 min at room temperature with shaking.**

↓

Step 6.	**Aspirate, wash 2 times for 1 min at room temperature with shaking.**

↓

Step 7.	**Stick dry strips onto the Evaluation protocol.**

16. Specification and diagnostic importance of the antigens for the kit LIA VIDITEST anti-Borrelia IgM garinii

antigen	specification
OspC *B. garinii* (p25) (outer surface protein C)	outer surface protein C, IgM marker, major early antigen (stage of infection I., rarely II.)
FlaB *B. garinii*(p41) (Flagellin B)	Flagellin (internal fragment), outer surface flagella protein, early antigen for the IgM antibody response (stage of infection I.), may be non-specific (cross-reactivity with other spirochetes and flagellated bacteria)
DbpA *B. garinii* (Osp17, p17) (decorin- binding protein A)	decorin- binding host cell protein, early and late antigen, highly specific for IgM and IgG antibody response, stage of infection II. and III.
BmpA *B. garinii* (p39) (Borrelia membrane protein A)	membrane protein A, glykosaminopeptide receptor, late antigen for IgM and IgG antibody response), stage of infection II.- III.
OspA-LT *B. garinii* (p31) (outer surface protein A)	outer surface membrane lipoprotein, early antigen for the IgM antibody response, less sensitive, species-specific
OspC-GV *B. garinii* (p25) (outer surface protein C)	outer surface protein C, IgM marker, major early antigen (stage of infection I., rarely II.), species-specific

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