



LIA-VIDITEST anti-*Borrelia* IgG

ODZ - 316

Instruction manual

PRODUCER:

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1. TITLE:

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

2. INTENDED USE:

LIA-VIDITEST test kit is intended for qualitative detection of specific IgG 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 IgG antibodies are detectable 6 weeks after infection. The level of IgG antibodies usually increases with disease progression to the 2nd stage. During the 3rd stage of the disease the antibody levels in serum are high and can remain elevated for several years.

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.

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 immunodeficient, post-splenectomy or post-transplant patients. Anaplasma specific antibodies are detected in human serum by high-specific antigen p44.

3. TEST PRINCIPLE:

LIA-VIDITEST anti-*Borrelia* is confirmatory test for qualitative detection of specific IgG antibodies in human serum and 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 IgG 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 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 IgG 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 μ l 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-channell 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-IgG 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 14 antigen lines, as listed in table 1.

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

| | |
|--|--|
| IgM conjugate control | negative control |
| IgG conjugate control | positive control |
| cut-off control | give an interface between negativity and positivity |
| BmpA (p39) | mixture of antigens BmpA <i>B. afzelii</i> , <i>garinii</i> and <i>burgdorferi sensu stricto</i> , early and late antigen, highly specific |
| BBK32 | mixture of antigens BBK32 <i>B. afzelii</i> , <i>garinii</i> and <i>burgdorferi sensu stricto</i> , less used, highly specific |
| DbpA (Osp17) | mixture of antigens DbpA <i>B. afzelii</i> , <i>garinii</i> and <i>burgdorferi sensu stricto</i> , early and late antigen, highly specific |
| p83 | mixture of antigens p83 <i>B. afzelii</i> , <i>garinii</i> and <i>burgdorferi sensu stricto</i> , late antigen, highly specific |
| p58 | mixture of antigens p58 <i>B. afzelii</i> , <i>garinii</i> and <i>burgdorferi sensu stricto</i> , late antigen, specific |
| VlsE | species-conserved antigen, late antigen, highly specific |
| p35 (OspB) | species-conserved antigen, late antigen, specific |
| p21 (OspE) | species-conserved antigen, late antigen |
| p20 | species-conserved antigen, late antigen |
| DbpA-TT <i>B. afzelii</i> | early and late antigen, species-specific |
| DbpA-TT <i>B. garinii</i> | early and late antigen, species-specific |
| OspA-LT <i>B. afzelii</i> (p31) | early antigen, low sensitive, species-specific |
| OspA-LT <i>B. garinii</i> (p31) | early antigen, low sensitive, species-specific |
| p44 <i>Anaplasma phagocytophila</i> | highly specific antigen <i>Anaplasma phagocytophila</i> |

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:

- on all strips are intensive lines of IgG conjugate control and no or very weak lines of IgM conjugate control.
- 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 - |
| BmpA, BBK32, DbpA or DbpA-TT <i>B. afzelii</i> or DbpA-TT <i>B. garinii</i>, p83, VlsE | 5 | 3 | 0 |
| p58, p35, p21, p20, OspA-LT <i>B. afzelii</i> or <i>B. garinii</i> | 4 | 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 - 4 | negative |
| 5 - 7 | borderline |
| 8 - 45 | positive |

Evaluation of p44 antigen line (*Anaplasma phagocytophila*)

According to 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 100% and the specificity is 98%. Evaluation was performed with a set of blood samples that comprised of: a) anti-Borrelia IgG positive sera, b) anti-Borrelia IgG 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 5902 (positive control) | | | | | | | | | | | | | |
|------------|-----------|---|-------|-------|-------|--------|-------|-------|-------|-------|-----------------|-----------------|-----------------|-----------------|-----|
| strip code | gray zone | BmpA | BBK32 | DbpA | p83 | p58 | VisE | p35 | p21 | p20 | DbpA-TT afzelii | DbpA-TT garinii | OspA-LT afzelii | OspA-LT garinii | p44 |
| 002G01 | 229-234 | 130 | 189 | 147 | 232 | 243 | 186 | 237 | 240 | 235 | 241 | 229 | 235 | 235 | 239 |
| 002G02 | 228-233 | 132 | 188 | 158 | 233 | 244 | 170 | 236 | 244 | 238 | 245 | 234 | 239 | 239 | 246 |
| 002G03 | 230-235 | 136 | 189 | 146 | 238 | 246 | 192 | 240 | 246 | 240 | 244 | 237 | 242 | 239 | 243 |
| 002G04 | 227-232 | 132 | 190 | 153 | 234 | 245 | 172 | 238 | 244 | 240 | 245 | 236 | 241 | 243 | 244 |
| mean | 133 | 189 | 151 | 234 | 245 | 180 | 238 | 244 | 238 | 244 | 244 | 234 | 239 | 239 | 243 |
| SD | 2,517 | 0,816 | 5,598 | 2,630 | 1,291 | 10,708 | 1,708 | 2,517 | 2,363 | 1,893 | 3,559 | 3,096 | 3,266 | 2,944 | |
| v% | 2% | 0% | 4% | 1% | 1% | 6% | 1% | 1% | 1% | 1% | 1% | 2% | 1% | 1% | 1% |
| min | 130 | 188 | 146 | 232 | 243 | 170 | 236 | 240 | 235 | 241 | 229 | 235 | 235 | 239 | |
| max | 136 | 190 | 158 | 238 | 246 | 192 | 240 | 246 | 240 | 245 | 237 | 242 | 243 | 246 | |
| N | 4 | 4 | 4 | 4 | 4 | 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 5902 (positive control) | | | | | | | | | | | | | |
|------------|-----------|---|--------|-------|-------|--------|-------|-------|-------|-------|-----------------|-----------------|-----------------|-----------------|-----|
| strip code | gray zone | BmpA | BBK32 | DbpA | p83 | p58 | VisE | p35 | p21 | p20 | DbpA-TT afzelii | DbpA-TT garinii | OspA-LT afzelii | OspA-LT garinii | p44 |
| 008G02 | 219-224 | 139 | 177 | 125 | 223 | 240 | 121 | 228 | 240 | 234 | 246 | 234 | 240 | 240 | 239 |
| 008G20 | 222-227 | 148 | 190 | 127 | 227 | 238 | 144 | 229 | 239 | 234 | 241 | 236 | 240 | 239 | 235 |
| 007G05 | 232-237 | 187 | 217 | 158 | 239 | 245 | 159 | 237 | 245 | 239 | 244 | 239 | 246 | 243 | 246 |
| 007G20 | 220-225 | 159 | 183 | 138 | 231 | 242 | 157 | 229 | 241 | 231 | 243 | 233 | 240 | 240 | 236 |
| 002G09 | 216-221 | 129 | 176 | 141 | 226 | 239 | 181 | 232 | 239 | 232 | 240 | 229 | 235 | 237 | 236 |
| 004G35 | 230-235 | 148 | 192 | 151 | 236 | 243 | 154 | 239 | 243 | 241 | 246 | 239 | 242 | 241 | 241 |
| mean | 223-228 | 152 | 189 | 140 | 230 | 241 | 153 | 232 | 241 | 235 | 243 | 235 | 241 | 240 | 239 |
| SD | 20,017 | 15,118 | 12,992 | 6,186 | 2,639 | 19,704 | 4,633 | 2,401 | 3,971 | 2,503 | 3,847 | 3,564 | 2,000 | 4,167 | |
| v% | 13% | 8% | 9% | 3% | 1% | 13% | 2% | 1% | 2% | 1% | 2% | 1% | 1% | 1% | 2% |
| min | 139 | 177 | 125 | 223 | 238 | 121 | 228 | 239 | 231 | 241 | 233 | 240 | 239 | 235 | |
| max | 187 | 217 | 158 | 239 | 245 | 159 | 237 | 245 | 239 | 246 | 239 | 246 | 243 | 246 | |
| N | 6 | 6 | 6 | 6 | 6 | 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.

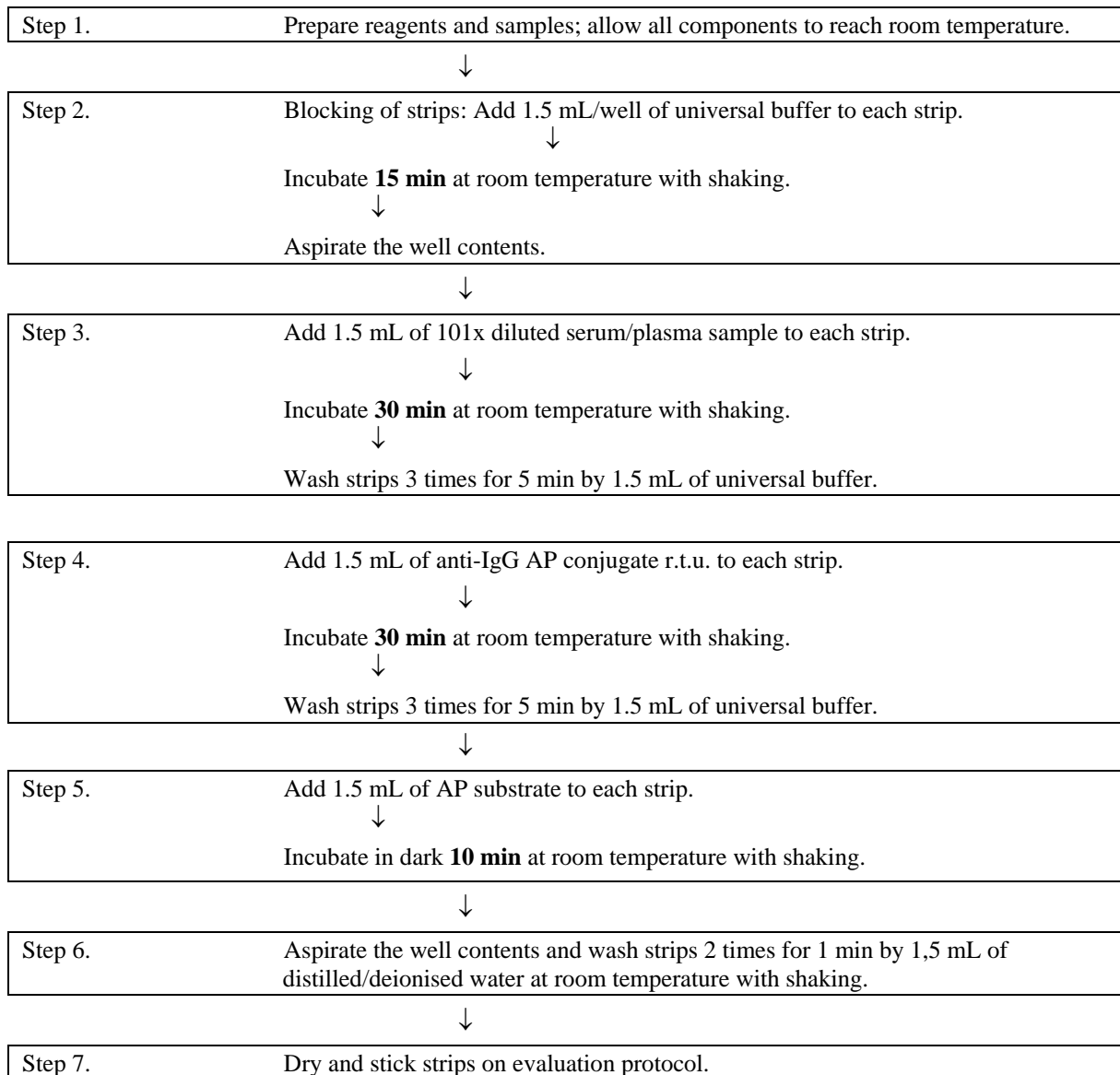
Kits are shipped in cooling bags. If you find damage at any part of the kit, please inform the manufacturer immediately.

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:



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