Borrelia VlsE IgG ELISA IgG Test Kit

Order-No.: EC023G00 (IgG Testkit)

Colour coding: gold/black

FOR IN VITRO DIAGNOSIS ONLY

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1. **Intended Use**

The Borrelia VlsE IgG ELISA, a high specific Screening Assay coated with recombinant VlsE-antigen of two genospecies (Borrelia burgdorferi sensu stricto and Borrelia garinii), is intended for the semiquantitative and qualitative detection of IgG-antibodies against Borrelia burgdorferi sensu lato in human serum.

2. **Diagnostic Relevance**

Lyme borreliosis (or Lyme disease) is a systemic disease which is caused by infection with the spirochaetes *Borrelia burgdorferi* (1,2). The spirochaetes are transmitted to humans from the bite of an infected tick. In Europe, the tick *Ixodes ricinus* has been identified as the main vector (5). At present, at least three human pathogenic *Borrelia burgdorferi* species are described for Europe (sensu lato), which are summarised under the term *Borrelia burgdorferi* sensu lato: *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii* (3,5,6).

*Lyme* borreliosis is a multisystem disease which develops in stages predominantly affecting the skin, the joints and the nervous system. Because of the wide range of clinical manifestations, the diagnosis of Lyme borreliosis is difficult (5). Among other things, a distinction between the different dermatological diseases (such as B-cell lymphoma of the skin and Lupus erythematosis), neurological diseases (such as multiple sclerosis) and internal diseases (such as arthritis and carditis) (15) is important for differential diagnostics.

The serological diagnostics of Lyme borreliosis is made more difficult by factors such as the following:

- a negative serology, particularly in the early stages, does not rule out Lyme borreliosis. The erythema migrans (primary stage) is seronegative in approximately 50% of cases (14)
- the formation of IgM antibodies can be completely absent
- IgM antibodies can persist over many months (10,11)
- IgG antibodies can still be detected years after clinical remission (10,11)
- cross reactions with other microorganisms have been observed (8,13). Diseases caused by bacteria, such as syphilis and herpes virus infections (particularly EBV), play an important role (12). False positive antibody responses can also occur in the presence of autoimmune antibodies (13).

*Lyme* borreliosis serology plays a supporting role in clarifying a clinically suspected case. Lyme borreliosis serology can supply important information about seronegativity or confirm a suspected case of fresh infection or advanced infection. However, it is essential that a positive antibody finding be assessed in association with the clinical picture (14).

We recommend carrying out the Lyme borreliosis serology in two stages (16). In the first stage, the samples to be tested should be examined with a sensitive screening test (MQ12/2000 recommends using an ELISA as a screening test). After this, equivocal and positive sera should be examined in a confirmation test (Line immunoassay/Western Blot). The analysis in the Western Blot enables the antibody response to individual pathogen antigens to be analysed specifically.

The latest development is in vivo-expressed antigens and these are also now available for diagnostic use. The special feature of these antigens is that they are only expressed in vivo by the borreliae in the infected mammal host (humans). Outstanding among these new in vivo-expressed antigens is the general genospecies protein VlsE (17, 18, 19). This acts as a second early marker next to OspC, particularly in IgG serology. Here, the tests have shown that, with early borrelioses, VlsE is frequently found in the IgG as well as the OspC in the IgM and that the sensitivity of the diagnosis of early Lyme borrelioses can be significantly increased.

3. **Test Principle**

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.
4. Package Contents

4.1 Package Contents (IgG Testkit)
1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. PBS-Dilution Buffer (blue, ready to use) 2x50ml, pH 7.2, with preservative and Tween 20
3. PBS-Washing Solution (20x concentrated) 50ml, pH 7.2, with preservative and Tween 20
4. IgG negative Control, 2000µl, human serum with protein-stabilizer and preservative, ready to use
5. IgG cut-off Control, 2000µl, human serum with protein-stabilizer and preservative, ready to use
6. IgG positive Control, 2000µl, human serum with protein-stabilizer and preservative, ready to use
7. IgG-Conjugate (anti-human), 11ml, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
8. Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml, ready to use
9. Citrate-Stopping Solution, 6ml, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

<table>
<thead>
<tr>
<th>Material</th>
<th>Status</th>
<th>Storage</th>
<th>Shelflife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Samples</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>max. 6h</td>
</tr>
<tr>
<td>Controls</td>
<td>Undiluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Microtitreplate</td>
<td>After Opening</td>
<td>+2 to +8°C (storage in the provided bag with desiccant bag)</td>
<td>3 months</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>Undiluted, After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Absorbent</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Conjugate</td>
<td>After Opening</td>
<td>+2 to +8°C (protect from light)</td>
<td>3 months</td>
</tr>
<tr>
<td>Tetramethylbenzidine</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Final Dilution (ready-to-use)</td>
<td></td>
<td>+2 to +25°C</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the Sekisui Virotech user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.
Always prepare patient-dilution freshly.
For a longer storage the sera must be frozen. Repeated defrosting should be avoided.
1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The Sekisui Virotech System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are parameter specific and only to use with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.

8.3 Virotech ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG-positive, negative and cut-off control as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera): for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350–400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme
8.4 Usage of ELISA processors
All Sekisui Virotech ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.
Sekisui Virotech recommends the following procedure:
1. Sekisui Virotech recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.
With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation
The ready to use controls serve for a semiquantitative determination of specific IgG antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control
a) OD-values
The OD of the blank should be < 0.15.
The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.
b) Virotech Units (VE)
The Virotech Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.
If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the Virotech Units (VE)
The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

\[
\begin{align*}
VE_{(positive\ control)} &= \frac{OD_{(positive\ control)}}{OD_{(cut-off\ control)}} \times 10 \\
VE_{(patient\ serum)} &= \frac{OD_{(patient\ serum)}}{OD_{(cut-off\ control)}} \times 10 \\
\end{align*}
\]

9.3 Interpretation Scheme IgG

<table>
<thead>
<tr>
<th>Result (VE)</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9.0</td>
<td>negative</td>
</tr>
<tr>
<td>9.0 - 11.0</td>
<td>borderline</td>
</tr>
<tr>
<td>&gt; 11.0</td>
<td>positive</td>
</tr>
</tbody>
</table>

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.

2. The cross-reaction between Borrelia and other spirochaeta may lead to a false positive result. Sera of patients with the following infections may cross-react: Syphilis (*Treponema pallidum*), Framboesia (*Treponema pertenue*), Recurrent Fever (Borrelia spec.), Leptospirosis (*Leptospira* spec.). Furthermore there may occur cross-reactions with Herpes Diseases (CMV, HSV, Parvovirus) (12, 13).

3. During the course of an EBV infection (infectious mononucleosis), the non-specific formation of anti-borrelia antibodies, particularly of the IgM class, may occur (12, 13).

10. Performance Data – DIAGNOSTIC

10.1 Analytical Sensitivity & Specificity

In order to determine the analytical sensitivity & Specificity 114 sera were tested on the Borrelia VlsE IgG ELISA and on the Borrelia *afzelii* + VlsE IgG EcoBlot.

<table>
<thead>
<tr>
<th>Borrelia VlsE IgG ELISA</th>
<th>B. <em>afzelii</em> + VlsE IgG EcoBlot</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>19</td>
</tr>
<tr>
<td>negative</td>
<td>0</td>
</tr>
<tr>
<td>borderline</td>
<td>6</td>
</tr>
<tr>
<td>positive</td>
<td>0</td>
</tr>
<tr>
<td>negative</td>
<td>74</td>
</tr>
<tr>
<td>borderline</td>
<td>12</td>
</tr>
</tbody>
</table>

This yields a analytical sensitivity of >99.9% for the Borrelia VlsE IgG ELISA. The analytical specificity of the Borrelia VlsE IgG ELISA is >99.9%.

The direct comparison to VlsE-bande yields a analytical sensitivity about 80%.

Borderline sera were not included in the calculation.

10.2 Prevalence (expected values)

The following table shows the examination of 78 blood donor sera:

<table>
<thead>
<tr>
<th>IgG</th>
<th>Anzahl</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>73</td>
<td>94</td>
</tr>
<tr>
<td>borderline</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>positive</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

This corresponds to a prevalence rate of 6%.

10.3 Intra-Assay-Variation Coefficient (Repeatability)

To determine the Intra-Assay-Variation Coefficient, 12 strips of different plates of one batch have been used for one test run. All 96 wells have been tested with one serum. The determined variation coefficient is < 9%.

10.4 Inter-Assay-Variation Coefficient (Reproducibility)

In 10 independent test runs, 3 positive sera were tested in different laboratories by different test persons. The determined variation coefficient is lower 15%.

11. References

16. Wilske et al. MQ12/2000; Urban&Fischer
### 12. Test Procedure Scheme

## Preparation of Patient Samples and Washing Solution

**▼ Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

**IgG-Samples – Dilution**

1:101
e.g.:

10 µl serum/plasma + 100 µl Dilution Buffer

(Serum Dilution Buffer is ready to use)

---

## Test Procedure

<table>
<thead>
<tr>
<th>Samples Incubation</th>
<th>30 minutes at 37°C</th>
<th>100 µl Patient Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>blank value (Dilution Buffer) and controls</td>
</tr>
<tr>
<td>Wash 4 times</td>
<td></td>
<td>400 µl Washing Solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Remove Residues on a Cellulose Pad</td>
</tr>
<tr>
<td>Conjugate Incubation</td>
<td>30 minutes at 37°C</td>
<td>100 µl Conjugate IgG</td>
</tr>
<tr>
<td>Wash 4 times</td>
<td></td>
<td>400 µl Washing Solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Remove Residues on a Cellulose Pad</td>
</tr>
<tr>
<td>Substrate Incubation</td>
<td>30 minutes at 37°C</td>
<td>100 µl Substrate</td>
</tr>
<tr>
<td>Stopping</td>
<td></td>
<td>50 µl Stopping Solution</td>
</tr>
<tr>
<td>Measure Extinctions</td>
<td></td>
<td>shake carefully</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Photometer at 450/620nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Reference Wavelength 620-690nm)</td>
</tr>
</tbody>
</table>