Enzyme immunoassays for the diagnosis of tick-borne encephalitis

EIA TBE Virus IgG and IgM kits are optimized and validated for detection of IgG and IgM antibodies in human serum, plasma and cerebrospinal fluid. EIA TBEV Ig kit is optimized and validated for detection of total antibodies in sera of all vertebrates (except mice).
INTRODUCTION

Tick-borne encephalitis is an endemic disease occurring in some parts of Europe and Asia. It is caused by a single-stranded RNA virus from the Flaviviridae family. The tick-borne encephalitis virus (TBEV) is transmitted mainly by ticks. A typical infection shows a two-stage course of the disease following an incubation period ranging from 4–20 days. First, a prodromal stage with flu-like symptoms develops (fever, strong headache, muscle ache, torpidity). After a decrease in non-specific symptoms, approximately 10% of cases include a second stage of infection in which neurological signs of the disease can develop (high temperature, severe headache, emesis, torpidity and meningo-encephalitic symptoms). An acute stage of tick-borne encephalitis lasts for 1–3 weeks. A severe course of the disease, with long-lasting ill effects, may occur in seniors.

DIAGNOSIS OF INFECTION

- Diagnosis of the disease is based on epidemiological anamnesis, clinical manifestation and laboratory tests
- Direct detection of the virus is not feasible for routine diagnostics
- Serology and examination of cerebrospinal fluid are the most important diagnostic tool of tick-borne encephalitis

DIAGNOSTIC VALUE OF DIFFERENT ANTIBODY CLASSES

**IgM:**
- Marker of acute infection
- Occasionally can persist up to 10 months after infection

**IgG:**
- Anamnestic or post-vaccination antibodies
- Persist for years ensuring protection against infection
- IgG avidity reflects the stage of infection

INTERPRETATION OF SEROLOGICAL RESULTS

<table>
<thead>
<tr>
<th>IgM</th>
<th>IgG</th>
<th>Interpretation</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>negative anti-TBEV antibodies</td>
<td>when suspecting acute infection test a new sample collected after some time (approx. 2 weeks)</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>past infection protective antibody titer after vaccination</td>
<td>when suspecting acute infection test a new sample collected after some time - monitor IgG antibody titer</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>early stage of acute infection</td>
<td>acute infection - IgG seroconversion after some time</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>acute infection recent vaccination</td>
<td>IgM antibodies may persist for more than 10 months after infection</td>
</tr>
</tbody>
</table>

RESULT INTERPRETATION AFTER VACCINATION

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP &lt; 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U &lt; 18 U/ml</td>
<td>negative anti-TBEV antibodies unfinished basic immunization</td>
<td>follow recommended vaccination schedule - if there is no seroconversion 4 weeks after the second dose, consider application of an additional dose; the third dose should be applied according to vaccination schedule</td>
</tr>
<tr>
<td>IgG +/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP = 0.9 – 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U = 18 - 22 U/ml</td>
<td>borderline anti-TBEV antibody titer</td>
<td>finished immunization - verify result by VNT; apply eventually a booster dose and check the antibody level after 2-4 weeks</td>
</tr>
<tr>
<td>IgG +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP &gt; 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U &gt; 22 U/ml</td>
<td>positive anti-TBEV antibodies seroconversion</td>
<td>follow recommended vaccination schedule</td>
</tr>
</tbody>
</table>
Anti-TBEV IgM antibodies are usually detectable after the prodromal stage. Anti-TBEV IgG antibodies are detectable simultaneously or a few days after the appearance of IgM antibodies.

POST-VACCINATION ANTIBODY RESPONSE
ELISA

TEST PRINCIPLE

The EIA TBE Virus IgG and IgM assays are based on a sandwich type of ELISA method.

Sandwich ELISA

The EIA TBEV Ig assay is based on a competitive type of ELISA method.

Competitive ELISA

ANTIGENS

Purified and inactivated native TBEV antigens.

CLINICAL APPLICATION

- Diagnostics of tick-borne encephalitis infection by detection of IgM and IgG specific antibodies against TBEV in serum and cerebrospinal fluid
- Monitoring and quantitative detection of post-TBEV vaccine antibody titre
- Monitoring total antibody titre in sera of all vertebrates (except mice) against TBEV in serum (EIA TBEV Ig)

SUMMARY PROTOCOL

<table>
<thead>
<tr>
<th>Step</th>
<th>Test steps</th>
</tr>
</thead>
</table>
| 1    | Dilution of samples  
• serum/plasma 1:101 (10 μl + 1 ml)  
• cerebrospinal fluid 1:2 (10 μl + 110 μl) |
| 2    | Incubate 30 min. at lab. temperature |
| 3    | Pipette Controls and diluted samples 100 μl  
• blank = 100 μl Sample diluent |
| 4    | Incubate 30 minutes at 37°C |
| 5    | Aspirate and wash the wells 5 times |
| 6    | Add Conjugate 100 μl  
• Including blank |
| 7    | Incubate 30 minutes at 37°C |
| 8    | Aspirate and wash the wells 5 times |
| 9    | Add 100 μl Substrate (TMB-Complete)  
• Including blank |
| 10   | Incubate 15 minutes at 37°C |
| 11   | Add 100 μl Stopping solution  
• Including blank |
| 12   | Read colour intensity at 450 nm |

EIA TBEV Ig has different EIA protocol (total assay time 2.5 hours).
ADVANTAGES

- Identical assay procedure
- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Short total assay time
- Avidity test available
- Sample diluent contains RF sorb (EIA TBE Virus IgM)
- Ready for automation
- Customer support

USER COMFORT

- Ready-to-use components
- Colour-coded components
- Interchangeable components
- Breakable colour-coded microplate strips
- CUT-OFF included
- Semiquantitative evaluation of results (Index of Positivity)
- Quantitative evaluation of IgG antibodies (U/ml)
- Conversion to Vienna units possible
- Easy assay procedure

CLINICAL DATA

ACUTE INFECTION – IgM AND IgG ANTIBODIES TITRES AND IgG AVIDITY

IgM antibody titer

IgG antibody titer

IgG avidity

Index of Positivity (IP)

Index of Positivity (IP)

Index of Avidity (%)

samples (n=47)

samples (n=47)

samples (n=47)
**ORDERING INFORMATION**

### ELISA

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBG096</td>
<td>EIA TBE Virus IgG</td>
<td>96</td>
</tr>
<tr>
<td>TBM096</td>
<td>EIA TBE Virus IgM</td>
<td>96</td>
</tr>
<tr>
<td>TBE096</td>
<td>EIA TBEV Ig</td>
<td>96</td>
</tr>
</tbody>
</table>

### KFR

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBEKFI</td>
<td>TBEV – CF – Ag lyophil. (1 ml)</td>
</tr>
<tr>
<td>KFA00</td>
<td>CF – AMBOCEPTORset (0.5 ml)</td>
</tr>
<tr>
<td>KFA01</td>
<td>CF – AMBOCEPTORset (1 ml)</td>
</tr>
<tr>
<td>KFC001</td>
<td>CF- COMPLEMENT (1 ml)</td>
</tr>
</tbody>
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**CONTACT**

TestLine Clinical Diagnostics Ltd.
Krizikova 68, 612 00 Brno, Czech Republic
Tel.: +420 549 121 203
Fax: +420 541 243 390
E-mail: sales@testlinecd.com
www.testlinecd.com

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.