

ENZYME IMMUNOASSAYS FOR THE DIAGNOSIS OF TICK-BORNE ENCEPHALITIS

Tick-Borne Encephalitis Virus (TBEV)

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:

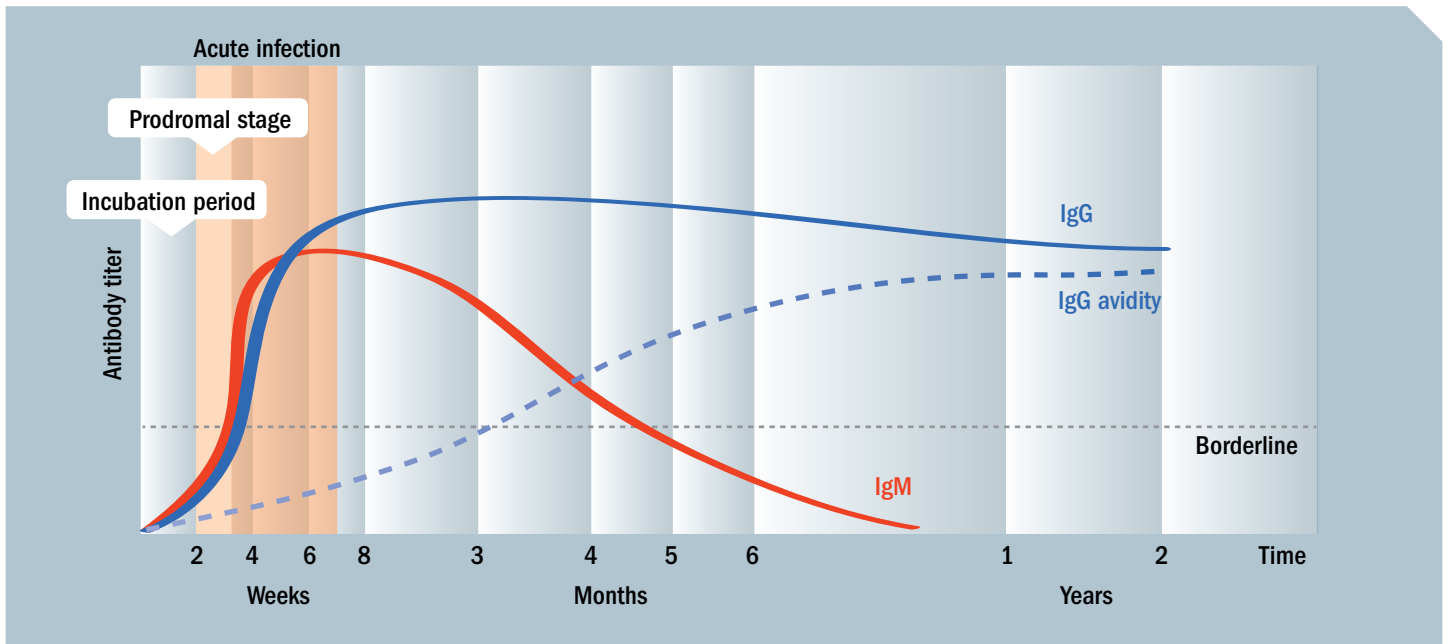
- ▶ Marker of acute infection
- ▶ Occasionally can persist up to 10 months after infection

IgG:

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

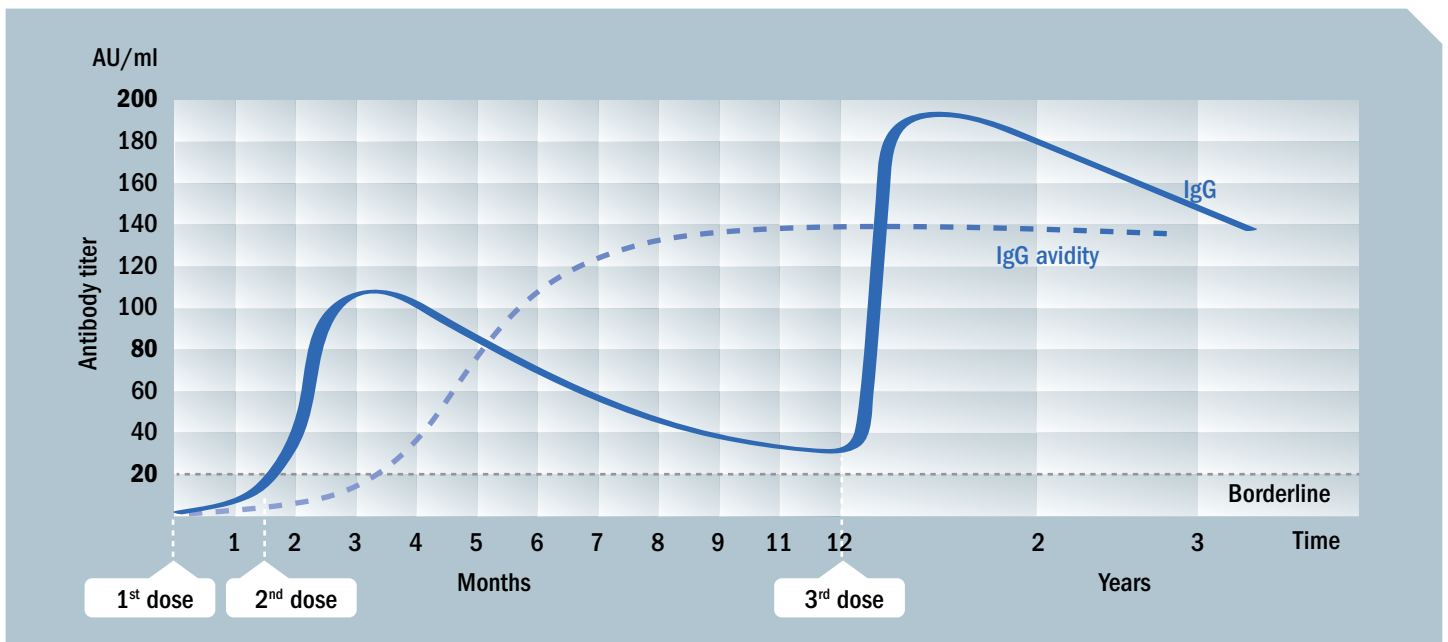
Antibody Response

Antibody response during TBEV infection



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

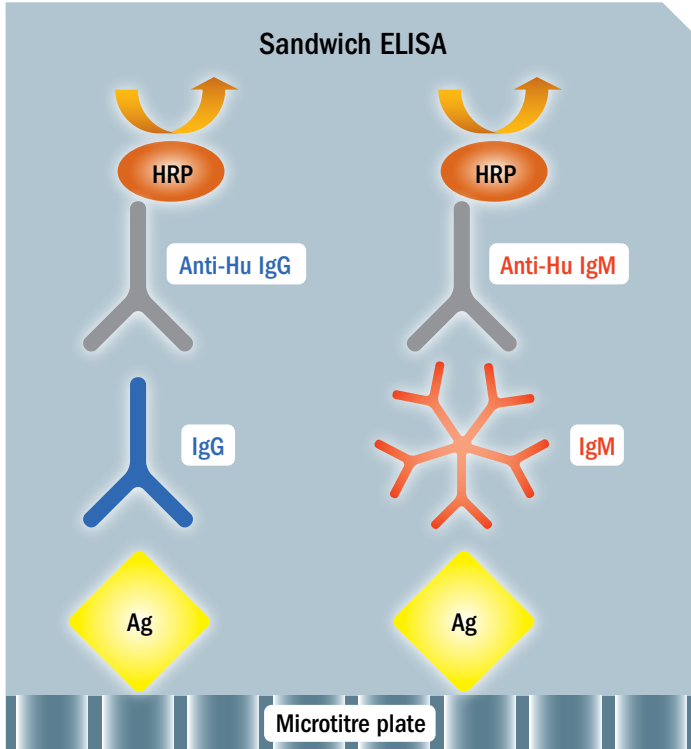


Clinical Application

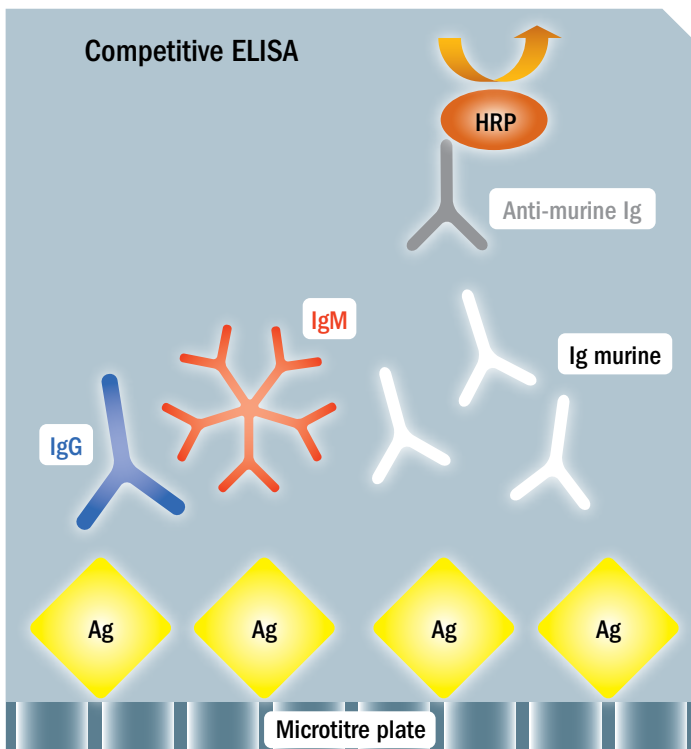
- ▶ Diagnostics of tick-borne encephalitis infection by detection of IgM and IgG specific antibodies against TBEV in serum and cerebrospinal fluid
- ▶ Monitoring and quantitative detection of post-TBEV vaccine antibody titre
- ▶ Monitoring total antibody titre in sera of all vertebrates (except mice)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

Test Principle

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



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



Summary of EIA Protocol

Step No.	Test steps
1	Dilute samples serum/plasma (1+100)
2	Incubate 10 minutes 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 100 µl Conjugate 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).

Antigens

Purified and inactivated native TBEV antigens.

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

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

Results Interpretation

IgM	IgG	Interpretation
+	-	acute infection (very early stage)
+	+	acute infection
-	+	anamnestic or post-vaccination antibodies
-	-	specific antibodies were not proven

Test Characteristics

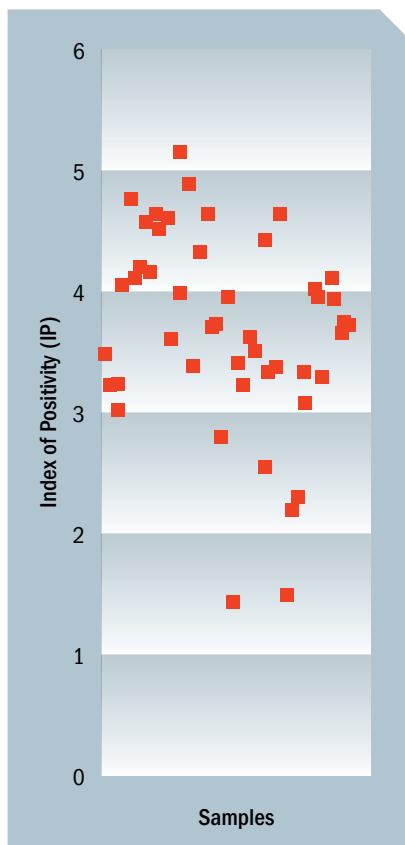
ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA TBE Virus IgG	98.7%	97.7%
EIA TBE Virus IgM	96.6%	98.9%
EIA TBEV Ig	95.7%	95.7%

Clinical Data

Acute infection – IgM and IgG antibodies titres and IgG avidity

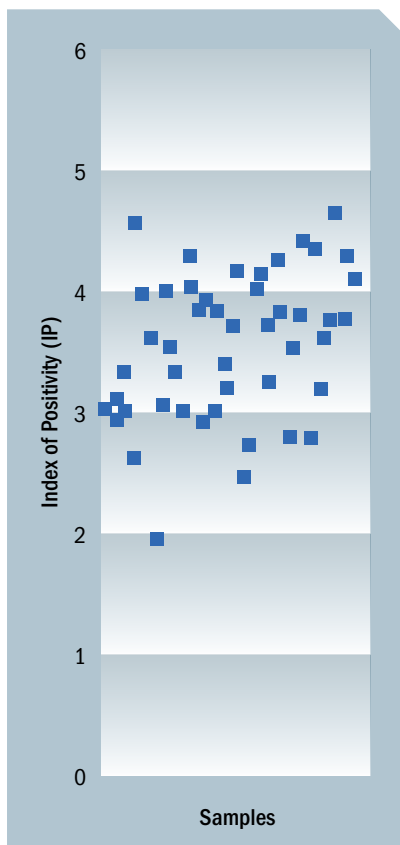
IgM antibody titer

acute infection (n = 47)



IgG antibody titer

acute infection (n = 47)



IgG avidity

acute infection (n = 47)

