**TBE IgG ELISA Kit**

**INTENDED USE**

Enzyme Immunoassay for Determination of IgG-Antibodies against TBE Virus in Human Serum, Plasma and Cerebrospinal Fluid (CSF). Control of humoral immune status and confirmation of seroconversion after vaccination. Identification of overt or latent TBE infection; usually with an additional anti-TBE-IgM determination. Antibody check following TBE infection. Confirmation of TBE vs Borreliosis following tick bite. Differential diagnosis of other CNS disorders.

**GENERAL DESCRIPTION**

In Europe, FSME (Tick-borne Encephalitis referred to as TBE) and Lyme Disease (Borreliosis) are the most frequent tick-borne infections. Borreliosis is very widespread but TBE is confined to special endemic regions. Both infections are similar in their development, consisting of two or more phases. The viraemic phase of TBE has an incubation period of 3-14 days with influenza-like symptoms in the first phase (1-8 days). After a nonfebrile interval of about one week the infection may enter into a second phase, characterised by neurological symptoms of varying intensity. This stage may last for many weeks. At the beginning of the second sickness phase, usually anti-TBE-IgM antibodies are detectable. Antibody levels reach their peak after 2-6 weeks. It can take 10 months for antibodies to fall below the level of detection. Anti-TBE-IgG antibodies are detectable simultaneously or a few days after the appearance of IgM-antibodies. Infection means immunity which mostly lasts a lifetime. Vaccination will also prevent the disease. Regular serological checks establish whether boosters are required. TBE-specific antibodies in Cerebrospinal Fluid (CSF) may be caused by a dysfunction of the hemato-encephalic barrier before or during an immune response to TBE antigens or may be the result of a local immune response. The antibody level fluctuations in the CSF may differ from those prevalent in serum/plasma. With FSME IgM and FSME IG it is possible to differentiate between specific TBE antibodies of the IgG and the IgM classes. Rheumatic factors and specific IgG do not interfere with IgM determination due to the addition of the Rf/IgG-absorber. The combination of both test systems allows the determination of the humoral immune status following TBE-vaccination or infection (IgG), early diagnosis of primary infection with TBE (IgM) and monitoring of changes of antibody levels (IgM/IgG) in human serum, plasma and cerebrospinal fluid.

**PRINCIPLE OF THE TEST**

FSME IgG is a two-step ELISA. Test wells in the ELISA test strips are coated with inactivated TBE virus. Diluted serum, plasma or cerebrospinal fluid samples are incubated in the test wells of the test strips. During the incubation period specific antibodies against the TBE virus are bound to the solid phase. Non-specific components are washed away. Conjugate reaction takes place during the second incubation phase. The anti-human-IgG peroxidase conjugate acts as a marker for the bound anti-TBE-IgG antibodies. Unbound conjugate is removed by a second washing step. In the third incubation phase the substrate reaction takes place. The peroxidase is part of the conjugate and oxidises the substrate tetramethylbenzidine (TMB) into a blue coloured substance. To stop the reaction sulphuric acid is added and the colour will change to yellow. The colour intensity is directly proportional to the anti-TBE-IgG-antibody concentration. The optical density is measured at a wavelength of 450 nm using an ELISA reader. Using the reference curve or the IMMNOZYM One Point Calibration anti-TBE-IgG antibodies can be quantitatively evaluated.

**REAGENTS PROVIDED**

MTP: 12 ELISA test strips, with 8 single break wells each, coated with inactivated TBE virus, sealed in an aluminium bag (again lockably) with desiccant.

WASH 10x: Wash buffer concentrate (10x), 0.1 M Tris/HCl pH 7.4, containing detergent, 0.01% (w/v) thimerosal, 1 bottle containing 100 ml. Dilute before use!

DIL: Incubation buffer, 0.01 M Tris/HCl, pH 7.4, containing detergent, 0.005% (w/v) thimerosal, coloured red, 2 x 75 ml.

CAL 1, CAL 2, CAL 3, CAL 4, CAL 5: Calibrators 1-5, human sera with stabilizers and preservatives, 1 bottle each, containing 0.35 ml per bottle. Concentrations are lot-specific as indicated on the bottle labels. Dilute before use!

POS LL, POS HL: Positive control sera, LL, “Low Level”, HL, “High Level”; for testing accuracy, human sera with stabilizers and preservatives, 1 bottle each containing 0.35 ml per bottle. Nominal values are lot-specific as indicated on the labels. Dilute before use!

CON: Conjugate, anti human IgG peroxidase, coloured blue, 1 bottle containing 0.6 ml. Dilute before use!

S: Substrate TMB (tetramethylbenzidine) in methyhypprolidone, 2 x 12 ml.

STOP: Stop solution, 0.5 M sulphuric acid, 1 bottle containing 15 ml.
Adhesive foil; for covering ELISA test strips, 2 pieces.
Evaluation sheet for plotting reference curve, 2 pieces.
Patient protocol, 1 piece.
Evaluation sheet for One Point Calibration, lot-specific, 1 piece.
Reference curve for One Point Calibration, lot-specific, 1 piece.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled water
2. Tubes for dilution of samples
3. Graduated cylinder (1000 ml)
4. Precision pipette (5 µl, 10 µl, 200 µl, 500 µl, and 1000 µl)
5. Pipettes (10 ml and 20 ml)
6. Multichannel pipette (50 µl, 250 µl)
7. Sample mixer
8. Timer
9. ELISA reader, 450 nm filter
10. Software “One Point Calibration” for evaluation using Microsoft Excel 5.0 or higher (software will be provided for free on request)

ASSAY PROCEDURE

1. Reagent And Sample Preparation
1) Before
Before starting the test, warm all the components required to room temperature (20-26°C)
2) Dilution
a. Dilution of calibrator 1-5, positive control sera and samples, 1+100. Example: 5 µl + 500 µl incubation buffer. Mix thoroughly! For the determination by reference curve calibrators 1-5 and control sera are needed. For determination by one point calibration instead of calibrators 1-5 use only calibrator 4 and control sera.
b. Dilution of CSF, 1+8
Example: 50 µl CSF + 400 µl incubation buffer.
3) Preparation of conjugate working solution, 1+100
Example for 8 wells: 20 µl conjugate + 2000 µl incubation buffer. Prepare immediately before needed! Ready to use solution is stable for 60 min at room temperature (20-26°C).
4) Preparation of wash buffer, 1+9
Example for 12 x 8 well strips: 30 ml wash solution concentrate (10x)+270 ml distilled water. Mix thoroughly! Ready to use solution can be stored at 2-8°C for 2 month.

2. Assay Steps
1) Pipette 200 µl diluted calibrator / control / sample per well. Calibrators should be placed in strips 1 and 2. Cover strips with adhesive foil. Incubate at room temperature (20-26°C) for 60 min.
2) Empty microassay strips and fill each well with 250 µl ready to use wash buffer. Empty wells again and repeat this wash step twice. Remove excess liquid by tapping the strips onto absorbent paper.
3) Pipette 200 µl ready to use conjugate per well. Cover with adhesive foil. Incubate at room temperature (20-26°C) for 60 min.
4) Empty microassay strips and carry out wash steps as described above (3x250 µl per well).
5) Pipette 200 µl ready to use substrate per well, incubate at room temperature (20-26°C) for 30 min (BEP III 20 min)
6) Pipette 50 µl of stop solution per well, shake for 10 sec, measure colour within 10 min at 450 nm (reference wavelength at 650 nm).

RESULTS
1. Vaccination
1) Anti-TBE-IgG antibodies negative
No latent immunisation before vaccination.
No seroconversion after vaccination. This may be the case after the first vaccination and, as an exception, also after the second and third vaccination or post-booster (no or low responders). If needed the basic immunisation should be completed. Success or failure of vaccination should then be established by serological testing.
2) Anti-TBE-IgG antibodies borderline level
This may be a case of seroconversion. Continue with basic immunisation or booster. Repeat anti-TBE-IgG test within 2-4 weeks. This may be a non-specific reaction.
3) Anti-TBE-IgG antibodies positive
This is a case of seroconversion.
Check case history data of vaccination and, if needed, complete basic immunisation or give a booster.
2. Infection
1) Anti-TBE-IgM antibodies and anti-TBE-IgG antibodies negative
In all likelihood there is no infection with the TBE virus. If such an infection is suspected repeat test within 7-10 days with new blood sample. A TBE infection may be either excluded or confirmed with a high degree of probability. A differential diagnosis for other infections of the CNS and, if there was a tick bite, borreliosis should be considered.
2) Anti-TBE-IgM antibodies negative and anti-TBE-IgG antibodies positive
There is either latent immunisation or the infection occurred weeks or months before. If such an infection is suspected, a test for anti-TBE-IgM should be performed. A fresh TBE infection may be either excluded or confirmed with a high degree of probability.
3) Anti-TBE-IgM antibodies positive and anti-TBE-IgG anti-bodies negative
A TBE virus infection is likely. After such an infection, IgM and subsequently IgG antibodies appear in the plasma. In the early phase of the infection the anti-TBE-IgG determination may at first be negative or reach borderline levels. A repeat test for anti-TBE-IgG (serum/plasma) within 7-10 days is recom-mended in order to detect any changes in antibody concen-trations.
4) Anti-TBE-IgM antibodies and anti-TBE-IgG antibodies posi-tive Most probably it is a case of TBE virus infection, provided there has not been any vaccination. The patient shows the typical symptoms of TBE but the intensity may vary. In case of borderline levels, blood has to be sampled again and the test repeated within 7-10 days. Any changes in antibody concentra-tions have to be monitored.

CALCULATION & EVALUATION

1. Determination by one point calibration.
The software “One Point Calibration” for evaluation using Microsoft Excel 5.0 will be provided on request. Open Excel file One Point Calibration

TBE IgG Sera. Using the lot specific package sheet Evaluation sheet for One Point Calibration fill in the cells: absorbance nominal value of calibrator 4, absorbance range of cali-brator 4, reference curve coefficients A, B, C and D, and ab-sorbance value of calibrator 4. Absorbance of calibrator 4 has to be in the indicated range. Insert identification and absorbance mean values of the control sera and samples into the prepared calculation sheet. The correction of the absorbance and the calculation of the concentrations will be done automatically.

2. Evaluation of anti-TBE-IgG Antibodies in CSF
The corrected absorbance (OD)450 nm of the CSF sample is interpreted:

OD < nominal value of cut-off 1  negative
nominal value of cut-off 1<OD<nominal value of cut-off 2  borderline
OD > nominal value of cut-off 2  positive.

ASSAY CHARACTERISTICS

Recovery of spiked serum samples: Variation of the theoreti-cal expected value is ≤8%.
For the intraserial imprecision (n, 6-12) a coefficient of varia-tion (CV) of <8% bades on concentrations and <7% based on optical density was determined.
For the interserial imprecision (n, 5) a VK <17% was deter-mined.

1. Specificity
A panel of 235 “healthy” patients with no fresh TBE Infection or immunisation known in the case history was tested with one lot in duplicate measurements. Two patients were classi-fied as false positive. The specificity as fraction of individuals without the disease who are tested negative is 99%.

2. Sensitivity
A panel of 151 naturally infected patients was tested with one lot in duplicate measurements. One patient was tested false-negative. The sensitivity as fraction of patients harboring the disease who are tested positive is 94%.

3. Establishing cut-off levels
Using a sample panel of different randomly selected samples (negative n = 91, immunised n = 68, infected n = 107) in stratified analysis empirical cut-off values for anti-TBE-IgG anti-bodies were compared with those based on the balanced Youden index. By applying this procedure, the empirical val-ues of 63 VIEU/ml as the lower limit and 126 VIEU/ml as the upper limit of a grey zone were confirmed. The sensitivity or specificity of the test outside of the borders of the grey zone was 97 or 99%.

4. Interferences
Haemolytic and lipaemic samples do not interfere with the test. Cross reactions of antibodies against other flaviviridae may occur.

REFERENCES