IMMUNOZYM FSME IgG ALL SPECIES

Enzyme Immunoassay for Determination of
IgG-Antibodies against TBE Virus in Animal Serum

Art. Nr. : 7701075
Content: 96 Determinations
Storage: at 2-8°C

For research use only!

Introduction

In Europe, FSME (Tick-borne Encephalitis referred to as TBE) and Lyme Disease (Borreliosis) are the most frequent tick-borne infections. TBE is confined to special endemic regions in Southern Germany, Thuringia, Austria, Switzerland, Hungary, Sweden, Czech, Slovakia, Croatia, Slovenia as well as some regions in Eastern Europe and Asia.

The primary hosts of the TBE virus are mammals living in the forest. Ticks transfer the TBE virus to different (domestic) animals as well. The IMMUNOZYM FSME IgG ALL SPECIES can be used for the detection of infections with TBE virus in animals.

Application

Characterization of TBE virus reservoirs for epidemiologic reasons [1,2].
Identifying an overt or latent TBE infection in animals.
Antibody check following TBE infection in animals.
Differential diagnosis of other CNS disorders in animals.

Test Principle

IMMUNOZYM FSME IgG ALL SPECIES is a two-step ELISA. Wells in the ELISA test strips are coated with inactivated TBE virus. Diluted serum samples are incubated in the wells of the test strips (**sample incubation**). 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 **(conjugate incubation)**. The Protein G 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 **TMB substrate reaction** takes place. The peroxidase is part of the conjugate and using the substrate hydrogen peroxide oxidizes the chromogen to a blue coloured substance. To stop the reaction, sulphuric acid is added and the colour changes 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. The calcuation can be performed quantitatively.

Materials and Reagents Provided

MTP, 12 ELISA test strips, with 8 single break wells each, coated with inactivated TBE virus, sealed in an aluminium bag with desiccant. Ready to use!

WASH 10x, Wash buffer concentrate (10x), 0.1 M Tris/HCl pH 7.4, containing detergent and preservative, 1 bottle containing 100 ml. Dilute before use!

CAL 1, **CAL 2**, **CAL 3**, **CAL 4**, **CAL 5**, Calibrators 1-5, lyophilized human sera with stabilizer and preservative, 1 bottle each. **Concentrations are lot-specific as indicated on the Quality Control Certificate.** Reconstitute and dilute before use!

POS LL, POS HL, Positive control sera, LL, “Low Level”, HL, “High Level”; for testing accuracy, lyophilized human sera with stabilizer and preservative, 1 bottle each. **Nominal values are lot-specific as indicated on the Quality Control Certificate.** Reconstitute and dilute before use!

CON 101x, Conjugate, Protein G peroxidase, coloured blue, 1 bottle containing 0.5 ml. Dilute before use!

TMB, Substrate tetramethylbenzidine; 2 x 12 ml. Ready to use!

STOP, Stop solution, 0.5 M sulphuric acid, 1 bottle containing 10 ml. Ready to use!

Adhesive foil; for covering ELISA test strips, 2 pieces.

Evaluation sheet for plotting reference curve, 2 pieces.

Materials and Reagents Required but not Provided

Distilled water
Tubes for dilution of samples
Graduated cylinder (1000 ml)
Precision pipette (5 µl,10 µl, 200 µl, 500 µl,1000 µl)
Pipettes (10 ml and 20 ml)
Multichannel pipette (50 µl, 250 µl)
Sample mixer, Timer
ELISA reader, 450 nm filter

Stability of Reagents

Store the test kit and components at 2-8°C. The unopened reagents are stable until the expiry date indicated on the label.

Stability after opening:

The **undiluted** test reagents are stable until the expiry date indicated: **MTP**, **CON 101x**, **STOP**, **WASH 10x**, **TMB**.

The stability of reconstituted and/or diluted reagents is shown below:

Stability after reconstitution:
Calibrators and controls: 2 weeks at 2-8°C or 6 month at -20°C.
Stability after dilution:
Calibrators and controls: 2 h at room temperature (20-26°C).
Wash buffer (ready to use): 6 weeks at 2-8°C.
Conjugate working solution: 60 min.

Test Performance

Preparations

Sample material: animal serum

Before starting the test, warm all the components required to room temperature (20-26°C).

Preparation of wash buffer, **1+9**: Example: Add 30 ml **WASH 10x** to 270 ml destilled water. Mix thoroughly! **Diluted washing buffer concentrate = working buffer.**

Reconstitution of calibrators and control sera: Reconstitute calibrators and control sera for 15 minutes with 200 µl working buffer and mix for 10 seconds (sample mixer). After reconstitution the solutions are clear to slightly turbid.

Dilution of calibrator 1-5, positive control sera and samples, **1+50**: Add 10 µl calibrator, control or sample to 500 µl **working buffer**. Mix thoroughly!

Preparation of conjugate working solution **(1+100)**: Prepare immediately before the sample incubation period is completed. Example for 8 wells: Mix 20 µl conjugate solution with 2000 µl working buffer.

Assay Procedure

SAMPLE INCUBATION (notes 1, 2, 3, 4)	diluted **calibrators** diluted **control sera** diluted **samples** to be pipetted into test wells cover test strips with adhesive foil	**200 µl**
	incubate at **room temperature**	**60 min**
WASH (note 5)	**working buffer**	**3 × 200 µl**
CONJUGATE INCUBATION (notes 1, 4)	pipette **conjugate working solution** into test wells cover test strips with fresh adhesive foil	**200 µl**
	incubate at **room temperature**	**60 min**
WASH (note 5)	**working buffer**	**3 × 200 µl**
TMB REACTION (notes 1, 4, 7)	pipette **TMB solution** into test wells	**200 µl**
	incubate at **room temperature**	**30 min**
STOP (note 7)	pipette **Stop solution** into test wells	**50 µl**
MEASURE (notes 6, 7)	ELISA reader, **450 nm**	measure within 10 min

Notes for the User

1. Reagents from different test lots must not be combined.
2. Sample storage: The samples should be fresh and can be stored for several months when undiluted and at a temperature of at least -20°C. Repeated freezing/thawing should be avoided.
3. Calibrators and control sera are HIV antibody, HCV antibody and HBsAg negative. Nevertheless, these components should always be considered as potentially infectious.
4. Precision and recovery, among others, depend on the following critical factors:

   Mix all diluted preparations for 10 seconds thoroughly using a sample mixer.

   Run tests in duplicates.

   Place calibrators in strips 1 and 2.

   Performe the incubations at room temperature (20-26°C).

   Maintain an exact pipetting sequence and timing of all incubations.

   - Incubation periods for samples, conjugate and TMB as indicated should always commence after the last pipetting step. Incubation periods should not be exceeded by more than 10%.
   - During the sample incubation, the time to pipette the diluted calibrators, control sera and samples should not exceed 60 seconds for each ELISA test strip (8 wells).
   - During the conjugate incubation, TMB reaction and stopping procedure, the time to pipette the TMB and stop solution should not exceed 10 seconds for each ELISA test strip. Short pipetting times are achieved by using a multichannel pipette.
   - **Important note**: During the incubation steps the microtiter strips must not be agitated.
5. After the last washing operation the test wells must be carefully drained by aspiration and tapping on absorbent paper.
6. The test precision may be improved by also measuring at 620 nm or 690 nm and using the differences of absorbance at 450 nm and 620 nm or at 450 nm and 690 nm for test evaluation.
7. Stop solution (sulphuric acid) and components of TMB may cause skin irritations. If acid or TMB should come into contact with eyes, rinse it out immediately with plenty of water and consult a physician!
8. Some of the reagents contain preservatives (e. g. ProClin®). Do not swallow! Avoid any contact with skin or mucous membranes!

Test Evaluation

Establishing the reference curve

Use evaluation sheets provided in the package:

x-axis: Concentrations in VIEU/ml[i]
y-axis: Absorbance (optical density)

Use mean values of calibrators, control sera and sample measurements each.

Plot the mean values of calibrators on the evaluation sheet and connect points with a curved ruler. If evaluation software is used, a programme for multiple and non-linear regression (e. g. multiple-parametric curve adaptation) is recommended.

Quality control

The absorbance of calibrator 1 should not exceed 0.5.

The absorbance of the highest calibrator should be higher than 1.0.

The difference in the absorbance of calibrators 5 and 1 should be at least 1.0.

Concentrations of control sera may be taken from the reference curve. The control levels obtained are used to check whether evaluation is correct.

Determination of sample concentration

Concentrations of the sample may be taken from the reference curve.

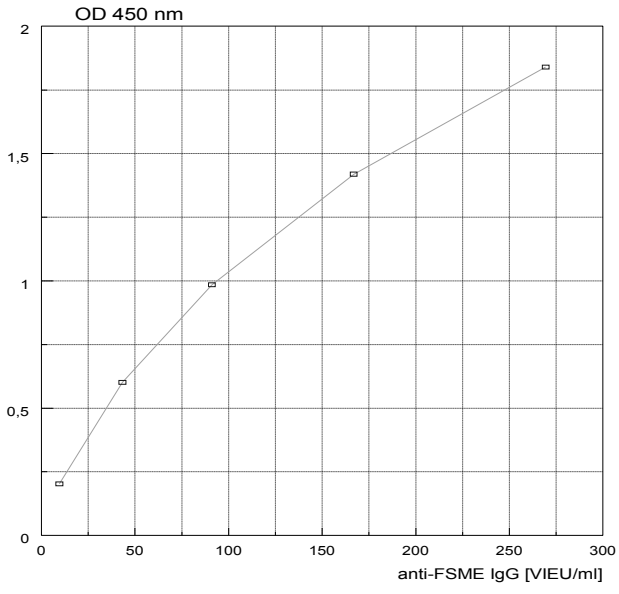
Samples with an absorbance exceeding that of calibrator 5 should be prediluted (1+1) with working buffer. The concentration thus obtained has to be multiplied by the dilution factor 2.

Evaluation of the results obtained by measurements

The results of the test can be interpreted according to the following table:

VIEU[i] / ml	anti-TBE-IgG antibodies
< 63	negative
63 - 126	borderline
> 126	positive

Fig. 1: Example of Reference Curve



Test Characteristics

Intra-assay imprecision (within run imprecision): Three human samples with concentrations ranging from 20-340 VIEU/ml were measured 12 times each in duplicate. Intra-assay imprecision related to concentrations ranged between 6% and 8%.

Inter-assay imprecision (between run imprecision): Three series of 28 human samples with concentrations from
20 -250 VIEU/ml, all in one lot, were measured in duplicate. Inter-assay imprecision related to concentrations rated between 2% and 25%. The mean value of imprecisions was below 10%.

Establishing cut-off levels: Human test samples were selected at random (negative n = 91, immunised n = 68, infected n = 107), using a stratified procedure. Empirical cut-off values (Prof. Ch. Kunz/Vienna) for anti-TBE-IgG antibodies were compared with those based on the Youden index [8].

By applying this procedure, the empirical values of 63 VIEU/ml as the lower limit and 126 VIEU/ml as the upper limit of a borderline zone were confirmed. The sensitivity and specificity of the test outside the borderline zone was 97% and 99%, respectively.

Interferences: Haemolytic and lipemic samples do not interfere with the test. Cross reactions of antibodies against other flaviviridae (e. g. Dengue Virus, Yellow fever Virus, West Nil Virus) may occur.

References and Recommended Reading

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[i] **VIE**NNA **U**NITS (according to Professor Ch. Kunz/Vienna)

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