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TBE/FSME vet ELISA

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CONTENTS

1. INTRODUCTION

Tick Born Encephalitis (TBE) is caused by a virus that belongs within the genus flavivirus in the Flaviviridae family. This is a human pathogenic single stranded RNA virus. It is usually transmitted by tick bites or via raw milk from infected animals. Three subtypes can be distinguished:

- Far Eastern Subtype: It is found mainly in Russia, east of the Urals and in parts of China, Japan and Korea. Host of this subtype is the *Ixodes persulcatus*.
- Western subtype: occurrence in Central, Eastern and Northern Europe. In Germany there are special areas, like Baden-Württemberg, Bavaria, and Hessen. Host of this subtype is *Ixodes ricinus*.
- Siberian subtype

From the veterinary point of view the following animal species are of importance:

Species	Importance
Small mammal populations, particularly mice	They represent the primary reservoir of pathogens, since they contribute through their long viremic phase significantly to virus maintenance and virus spread in the habitat.
Goats, cows and sheep	Infected animals are excreting the virus during their viremic phase via the milk and may represent a source of infection for humans.
Dogs and horses	TBE infections can cause serious neurological symptoms. The disease starts with high fever. It can cause behavioral change and seizures. Several failures of the cranial nerves can be observed. Usually non-specific symptoms occur and therefore a differential diagnosis with other neurological diseases and the detection of specific antibodies is useful.
Farm and wild animals such as goats, sheep, cattle, horses, foxes and various small mammals	They are sentinels for the characterization of local natural foci by antibody detection assays.

Infections may be diagnosed:

- Direct detection by real time RT-PCR
- Serology, using antibody detection ELISA
- Serum neutralization test (SNT)

2. INTENDED USE

The TBE/FSME vet ELISA is intended for the quantitative determination of antibodies against TBE/FSME in veterinary serum.

3. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

1. **SORB MT Microtiterplate:** 12 break-apart 8-well snap-off strips coated with TBE/FSME antigens; in resealable aluminium foil.
2. **SAM DIL Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).
3. **STOP SOLN Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
4. **WASH SOLN 20x Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
5. **ENZ CONJ Conjugate:** 1 bottle containing 20 mL of peroxidase labelled Protein A/G; coloured yellow; ready to use; white cap; ≤ 0.02% (v/v) MIT.
6. **SUB TMB TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
7. **CAL A – E Standards:** 5 vials, each containing 2 mL; coloured yellow; ready to use*;* ≤ 0.02% (v/v) MIT.

Standard A:	0	U/mL; blue cap	
Standard B:	50	U/mL; green cap	
Standard C:	130	U/mL; yellow cap	[U = Units]
Standard D:	200	U/mL; red cap	
Standard E:	300	U/mL; white cap	

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate wells
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with TBE/FSME antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use veterinary mammalian serum samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with Sample Dilution Buffer. Dispense 10 µL sample and 1 mL Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.
Perform all assay steps in the order given and without any delays.
A clean, disposable tip should be used for dispensing each standard/control and sample.
Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.
If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.
Bichromatic measurement using a reference wavelength of 620 nm is recommended.
Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank: Absorbance value < 0.100**
- **Standard A: Absorbance value < 0.200**
- **Standard B: Absorbance value > 0.050**
- **Standard C: Absorbance value > Standard B**
- **Standard D: Absorbance value > Standard C**
- **Standard E: Absorbance value > 1.000**

Standard A < Standard B < Standard C < Standard D < Standard E
If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

In order to obtain **quantitative results in U/mL** plot the (mean) absorbance values of the 5 Standards A, B, C, D and E on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 50, 130, 200 and 300 U/mL) and draw a standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis).
Read results from this standard curve employing the (mean) absorbance values of each sample.
For the calculation of the standard-curve mathematical Point to Point function should be used.

9.3. Interpretation of Results

Normal value ranges for this ELISA should be established by each laboratory based on its own sample populations in the geographical areas serviced.
The following values should be considered as a guideline:

Positive	> 30 U/mL	Antibodies against the pathogen are present.
Equivocal	20 – 30 U/mL	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 20 U/mL	The sample contains no antibodies against the pathogen..

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.
The performance data have been established with serum samples from dogs, sheeps, goats, horses, deers, and wild boars. Due to the nature of the Protein A/G conjugate this ELISA should react with other mammalian species also. More detailed information is available on request.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	1.411	3.51
#2	24	1.991	2.23
#3	24	1.913	8.93

Interassay	n	Mean (U)	CV (%)
#1	12	26.55	9.18
#2	12	15.38	12.99
#3	12	253.81	10.48

10.2. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
Specificity: 97.37 % (95 % confidence interval: 86.19 % - 99.93 %)

10.3. Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
Sensitivity: 100.0 % (95 % confidence interval: 78.20 % - 100.0 %)

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Cross reactions cannot be excluded.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- **Only for research use.**
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

BIBLIOGRAPHY

Roggendorf M. Frühsommer-Meningoenzephalitis – Wer soll geimpft werden? Therapiewoche, 40, 1173 (1990)
Roggendorf M. et al. Serological Diagnosis of Acute Tick-Borne Encephalitis by Demonstration of Antibodies of the IgM class. J.Med.Virol., 7, 41 (1981)
Hofmann H. et al. Rapid diagnosis of Tick-Borne Encephalitis by Means of Enzyme Linked Immunosorbent Assay. J.Gen.Virol. , 42, 505 (1979)
Hofmann H. et al. ELISA for IgM Antibodies against Tick-Borne Encephalitis Virus: Quantification and Standardization of Results. Zbl.Bakt.I.Orig., 255, 448 (1983)
Tick-Borne Encephalitis (TBE) and its Immunoprophylaxis, IMMUNO AG, Wien (1997)

Epidemiologisches Bulletin, 2011/Nr. 17; FSME: Risikogebiete in Deutschland (Stand: April 2011); Robert Koch-Institut, Berlin

Epidemiologisches Bulletin, 2015/Nr. 21; FSME: Risikogebiete in Deutschland (Stand: Mai 2015); Robert Koch-Institut, Berlin

Amicizia, Daniela, et al. "Epidemiology of tick-borne encephalitis (TBE) in Europe and its prevention by available vaccines." *Human vaccines & immunotherapeutics* 9.5 (2013): 1163-1171.

Donoso Mantke O, Escadafal C, Niedrig M, Pfeffer M: Working group for tick-borne encephalitis virus. Tick-borne encephalitis in Europe, 2007 to 2009. Eurosurveillance 2011;16:

ABBREVIATIONS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY
TBE/FSME vet ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Sub-strate Blank (A1)	Standard A	Standard B	Standard C	Standard D	Standard E	Sample (diluted 1+100)
Standard A	-	100 µL	-	-	-	-	-
Standard B	-	-	100 µL	-	-	-	-
Standard C	-	-	-	100 µL	-	-	-
Standard D	-	-	-	-	100 µL	-	-
Standard E	-	-	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37±1 °C** Wash each well three times with 300 µL of Washing Buffer							
Conjugate	-	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20…25°C) Do not expose to direct sunlight Wash each well three times with 300µL of Washing Buffer							
TMB Sub-strate Solu-tion	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20…25°C) in the dark							
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)							

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta