

FSME / TBE ELISA IgG / IgM Test Kit

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EC117.00 (IgG / IgM Test Kit)

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FOR IN VITRO DIAGNOSIS ONLY

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1. Intended Use

The TBE ELISA is intended for the detection of an acute or recent infection with Tick-Borne-Encephalitis-Virus (TBE) or for the detection of vaccination antibodies.

2. Diagnostic Relevance

The TBE virus belongs to the family of the flaviviruses (2). It is distributed mainly in Central and Eastern Europe: Russia, Latvia, southern Sweden, the Czech Republic, Slovenia, Austria, Switzerland, Croatia and Albania. In Germany, endemic regions with natural foci are found mainly in the southern part of the country: Bavaria, Baden Württemberg; rarely Odenwald, east of Marburg, Rhineland-Palatinate, Saarland, Thuringia, Saxony and very rarely Brandenburg (3, 4).

It is usually transmitted to humans by the bite of infected ticks (*Ixodes ricinus*, *Ixodes persulcatus*). Transmission from human to human does not occur (5).

The TBE virus is the cause of early summer meningoencephalitis. The disease has a biphasic course. After an incubation period, usually of 7 – 14 days (rarely 3 – 28 days), the viraemic phase (first phase), which lasts 3 – 7 days, begins with flu-like symptoms such as headache and arthralgia, fever, catarrhal upper respiratory tract symptoms and sometimes gastrointestinal complaints. If the course is abortive, the disease ends after the viraemia (2).

In about 10% of cases, the second phase begins after a usually symptom-free interval of approximately 8 days with a further marked rise in fever. This can manifest itself in the course of the disease as meningitis, meningoencephalitis or meningoencephalomyelitis. The mortality rate in severe forms of the illness is 1 – 2% (2).

To determine the level of immunity, the ELISA is the test format of choice, as the CFR is nonspecific and not sufficiently sensitive, and NT or HAT are not suitable for detecting IgM antibodies (2).

About 10 days after the onset of the symptoms of the first phase, the IgM antibody level starts to rise (1), and reaches its peak in the early stage of the disease in about the sixth week (2). After about 10 months, the IgM antibodies fall to levels that can no longer be detected (2).

The IgG antibody level rises from about the 14th day after the start of the disease (1). After approximately 6 weeks, the IgG antibodies reach their maximum level (6), which persists for several years or falls only slightly (2).

Immunity can be built up by immunisation but is limited to a few years and must be supplemented by regular boosters (5).

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents IgG and IgM Test Kit

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
7. **IgM negative Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelf life of the Test kit and the ready to use reagents

Store the test kit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelf life
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitre plate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA hand washer or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the Sekisui Virotech user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The Sekisui Virotech System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 Virotech ELISA Test Procedure

1. For each test batch, pipette 100 µl each of the ready-to-use dilution buffer (blank), the controls and the diluted patient sera. We recommend that the blanks, controls and patient sera should be in duplicate. It is absolutely essential that the cut-off's control should be in duplicate. Working dilution for the patient sera: 1+100; e.g. 10 µl serum + 1 ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All Sekisui Virotech ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

Sekisui Virotech recommends the following procedure:

1. Sekisui Virotech recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgM-antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) Virotech Units (VE)

The Virotech Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the Virotech Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$\text{VE (positive control)} = \frac{\text{OD (positive control)}}{\text{OD (cut - off control)}} \times 10$
$\text{VE (patient serum)} = \frac{\text{OD (patient serum)}}{\text{OD (cut - off control)}} \times 10$

9.3 Interpretation Scheme IgG and IgM

Result (VE)	Evaluation	Interpretation
< 9,0	negative	No antibodies detectable
9,0 – 11,0	borderline	No significantly increased antibody concentration repeat test, if necessary obtain 2nd sample
> 11,0	positive	Significantly increased antibody concentration IgM: <ul style="list-style-type: none"> • Acute Infection • Recent Infection • Vaccination antibodies IgG: <ul style="list-style-type: none"> • Recent Infection • Past Infection • Vaccination antibodies

Notes

1. As the TBE ELISA cannot differ between vaccination antibodies and antibodies built after infection the vaccination management should be followed.
2. The TBE ELISA may show cross reactivities to other flaviviruses. Therefore it may also react positive after Dengue virus infection or yellow fever vaccination.
3. It is possible that chronically TBE infected patients show an increased IgM titer without detectable IgG titer for years (7).

9.4 Limits of the Test

The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.

The TBE ELISA may show cross reactivities to other flaviviruses. Therefore it may also react positive after Dengue virus infection or yellow fever vaccination.

10. Performance Data

10.1 Diagnostic Sensitivity

124 clinically characterised sera from patients with TBE were tested in IgG and IgM to determine the diagnostic sensitivity. 123 sera were recognized as positive, one serum had a borderline result.

Under consideration of the overall finding, the diagnostic sensitivity for IgG and IgM is >99,8%. The borderline result was not considered.

10.2 Analytical Sensitivity

Three different test methods were used as basis to determine the analytical sensitivity: HAT, neutralisation test (NT) and ELISA.

142 TBE positive sera were retested in the HAT and compared with the Virotech ELISA. The borderline titer was 1:10, results below 1:10 were assessed as negative.

Sera collective (n=142)		TBE ELISA IgG and IgM overall result		
		Negative	Borderline	Positive
HAT Analytical Finding	Negative	1	-	6
	Borderline	-	-	-
	Positive	3	-	132

In reference to the HAT the sensitivity is 97,8%.

26 TBE positive sera were tested in the neutralisation test and compared with the Virotech ELISA. The borderline index for assessment of the neutralisation test (NT) was 1,0. Values below 1,0 were assessed as negative.

Sera collective (n=26)		TBE ELISA IgG and IgM overall result		
		Negative	Borderline	Positive
Neutralisation test (NT) Analytical finding	Negative	-	1	1
	Borderline	-	-	-
	Positive	-	-	24

In reference to the neutralisation test the sensitivity is >99,8%.

Borderline results were not considered.

28 TBE positive sera were tested in one ELISA and compared with the Virotech ELISA.

Sera collective (n=28)		Virotech TBE ELISA IgG		
		Negative	Borderline	Positive
ELISA Analytical finding	Negative	-	2	4
	Borderline	-	-	-
	Positive	1	-	21

The thus resulting sensitivity is 95,5%. Borderline results were not considered.

Sera collective (n=28)		Virotech TBE ELISA IgM		
		Negative	Borderline	Positive
ELISA Analytical Finding	Negative	-	-	-
	Borderline	-	-	-
	Positive	-	1	27

The thus resulting sensitivity is >99,8%. Borderline results were not considered.

10.3 Analytical Specificity

To determine the analytical specificity sera which have been regarded as TBE negative were tested in IgG and IgM. These sera were obtained from blood donors and routine tests.

Sera collective (n=187)	TBE ELISA IgG		
	Negative	Borderline	Positive
Negative	175	4	8

Sera collective (n=160)	TBE ELISA IgM		
	Negative	Borderline	Positive
Negative	158	2	0

The specificity for IgG is 95,6% and for IgM >99,8% for the TBE ELISA. Borderline results were not considered in the calculation.

10.4 Prevalence (Expected Values)

The following table shows the results of the examination of blood bank sera:

	IgG (n=80)	IgM (n=80)
Negative	73	80
Borderline	0	0
Positive	7	0

This corresponds to a prevalence rate of 8,8% in IgG and 0% in IgM. In IgG this may be vaccination antibodies.

10.5 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested with the same serum sample. The determined variation coefficient is < 12%.

10.6 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 10 independent test runs by different persons in different laboratories. The determined variation coefficient is < 15%.

11. Literature

1. VioMecum, Früh-Sommer-Meningo-Enzephalitis (FSME) (2003)
2. Blessing, J. in Virusdiagnostik, Diagnostische Bibliothek Band 1 (Hrsg.) Porstmann, T., Blackwell Wissenschafts-Verlag (1996)
3. Chiron Behring Homepage – Verbreitungsgebiete FSME (2003)
4. Baxter Deutschland GmbH – Zecken: FSME-Karte (2003)
5. RKI, Epidemiologisches Bulletin 16/99
6. Forsgren, M. et al., Intrathecal IgM, IgA and IgG antibody response in tick-borne encephalitis. Long-term follow-up related clinical course and outcome, Clin Diagn Virol 1997 Aug; 8 (2): 167-8.
7. Matveeva et al.: Antibodies against tick-borne encephalitis virus (TBEV) non-structural and structural proteins in human sera and spinal fluid, Immunology Letters, 46 (1995) 1-4

Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ **IgG-Samples – Dilution**
1:101

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

▼ **IgM-Samples - Dilution**
1:101

Rheumatoid factor-absorption with RF-SorboTech

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Test Procedure

