研究用試薬

Updates with respect to the previous version are marked in grey.

Anti-TBE Virus ELISA 2.0 (IgG)
Instructions for use

For in vitro diagnostic medical device IVD

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2661-9601-2 G	TBE virus	IgG	Ag-coated microplate wells	96 x 01 (96)

Intendend use

The test system provides semiquantitative or quantitative in vitro determination of human antibodies of the immunoglobulin class G against TBE virus in human serum or EDTA, heparin or citrate plasma to support the diagnosis of TBE virus infections. The product is designed for use by healthcare professionals and can be processed and evaluated manually or on automated instruments. The results should always be interpreted together with those of further laboratory diagnostic procedures and based on the clinical picture.

Clinical significance

The following information outlines the clinical background. The product may only be used for the indications specified in the chapter "Intended use".

By the current stand of knowledge, tick-borne encephalitis virus (TBEV, genus Flavivirus, family Flaviviridae) comprises the European (TBEV-Eu), Siberian (TBEV-Sib) and Far Eastern (TBEV-FE) subtypes. TBEV circulates between ticks, principally *Ixodes ricinus* (TBEV-Eu) and *Ixodes persulcatus* (TBEV-Sib, TBEV-FE), and small rodents [1-6]. Deer and other wild animals are important hosts of the adult ticks [1, 2, 5]. TBEV is transmitted via tick bites and, less frequently, by ingestion of raw milk and raw milk products from infected goats, sheep or cows [1-6]. Humans are accidental end hosts [5].

Most TBEV infections in humans remain asymptomatic. The average incubation time is 7 to 14 days and less if the infection is transmitted via foods. Symptomatic courses are mono- or biphasic, with differing severities depending on the virus subtype [3-5].

Following infection with TBEV-Eu, the disease is mostly biphasic. It starts with unspecific, flu-like symptoms. The first phase, from day 1 to 8, is the viraemic phase. After an asymptomatic interval of approximately one week, one third of the patients enters a second phase in which the virus penetrates the central nervous system. High fever and neurological manifestations such as meningitis, encephalitis and myelitis are typical symptoms. Long-time sequelae have been described in a smaller proportion of the severe courses. These encompass mainly pareses, but also seizures or headaches. The mortality amounts to 1 to 2% [1-7]. In children, the disease has a milder course and neurological sequelae occur less frequently than in adults [8].

Persisting infections accompanied by chronic (progressive) disease account for 1 to 3% of cases in Russia (TBEV-Sib). Infections with TBEV-FE lead to the most severe courses [3]. The mortality is given with 6 to 8% for TBEV-Sib infections and with up to 40% for TBEV-FE infections [4].

The vector ticks are found in many European and Asian countries. TBEV is only found in endemic regions. It has spread further over the past years. Most diseases occur in spring, early summer, and often also in autumn. In Europe and Asia, between 10,000 and 15,000 clinical cases are reported every year [1, 2, 4-6].

TBEV-specific IgM and IgG antibodies in serum and CSF can generally be detected in the second disease phase. IgM remains detectable for 6 to 7 weeks or longer. IgG persists life-long and protects from reinfection with TBEV [3-7].

The diagnosis is made based on the parallel detection of TBEV-specific IgM and IgG in serum or CSF or of a significant increase of the IgG concentration in two samples taken at an interval of 2 to 4 weeks, by means of an ELISA or immunofluorescence test. The sole detection of IgM is not sufficient. Diagnostics can be facilitated by taking into account the season as well as possible information on a stay in an endemic region. Following vaccination against TBEV, specific IgM can be detected over a prolonged period [4-7].

Cross-reactivity of anti-TBEV antibodies (especially those of class IgG) with other flaviviruses (yellow fever, dengue, Japanese encephalitis and West Nile fever viruses, also after vaccination) must be taken into account [5-7].

Several other viruses and bacteria (e.g. meningococcus) must be taken into account in differential diagnostics [5, 6].

Antigen

The microplate wells were coated with native, purified TBE virus antigens.

Test principle

The test kit contains microplate strips each with 8 break-off reagent wells coated with purified TBE virus antigens. In the first analysis step, the reagent wells are incubated with diluted patient samples. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. In a second analysis step, the bound antibodies are detected by incubating the samples with an enzyme-labelled anti-human IgG antibody (enzyme conjugate), which catalyses a colour reaction.

Contents of the test kit

Description	Colour	Format	Symbol
1. Antigen-coated microplate wells, 12 microplate strips each containing 8 break-off reagent wells in a frame, coated with TBE virus antigens (≤150 µl/well), ready for use	---	12 x 8	STRIPS
2. Calibrator 1 contains human serum/plasma with anti-TBE virus IgG (200 RU/ml) in buffer solution, ready for use Preservative: sodium azide <0.1% (w/w)	Red coloured in decreasing intensity.	1 x 2.0 ml	CAL 1
3. Calibrator 2 contains human serum/plasma with anti-TBE virus IgG (50 RU/ml) in buffer solution, ready for use Preservative: sodium azide <0.1% (w/w)		1 x 2.0 ml	CAL 2
4. Calibrator 3 contains human serum/plasma with anti-TBE virus IgG (20 RU/ml) in buffer solution, ready for use Preservative: sodium azide <0.1% (w/w)		1 x 2.0 ml	CAL 3
5. Calibrator 4 contains human serum/plasma with anti-TBE virus IgG (2 RU/ml) in buffer solution, ready for use Preservative: sodium azide <0.1% (w/w)		1 x 2.0 ml	CAL 4
6. Positive control contains human serum/plasma with anti-TBE virus IgG (≥22 RU/ml)* in buffer solution, ready for use Preservative: sodium azide <0.1% (w/w)	blue	1 x 2.0 ml	POS CONTROL
7. Negative control contains human serum/plasma with anti-TBE virus IgG (<16 RU/ml)* in buffer solution, ready for use Preservative: sodium azide <0.1% (w/w)	green	1 x 2.0 ml	NEG CONTROL
8. Enzyme conjugate peroxidase-labelled antibody against human IgG (<0.25 %) in buffer solution, ready for use Preservatives: ethanol <0.2% (w/w); 2-methylisothiazol-3(2H)-one hydrochloride <0.1% (w/w)	green	1 x 12 ml	CONJUGATE
9. Sample buffer buffer solution, ready for use Preservative: sodium azide <0.1% (w/w)	light blue	1 x 100 ml	SAMPLE BUFFER
10. Wash buffer buffer solution, 10x concentrate Preservative: sodium azide <0.1% (w/w)	colourless	1 x 100 ml	WASH BUFFER 10x
11. Chromogen/substrate solution TMB ≤0.04 % (w/w), H_2O_2 ≤0.05 % (v/v) in buffer solution, ready for use	colourless	1 x 12 ml	SUBSTRATE
12. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
13. Quality control certificate	---	1 protocol	QC CERTIFICATE

* Lot-specific reference values can be found on the quality control certificate.

Other materials and equipment required (not included in the test kit)

The list below shows instruments and materials that are required for the test performance, but not provided in the test kit. These articles can usually also be substituted by equivalent articles.

Description	EUROIMMUN order number
Microplate reader with evaluation software: wavelength of 450 nm, reference wavelength from 620 nm to 650 nm	YG 0415-0101-1
Optional: Automatic microplate washer: recommended. (Washing of the microplates can also be carried out manually.)	YG 0403-0101-1
Incubator: to incubate the microplate at +37 °C	YG 0431-0101
Calibrated pipettes	Not available from EUROIMMUN
Pipette tips	
Optional: Water bath: recommended to warm the wash buffer	
Distilled or deionised water	
Vibration shaker (vortex)	
Stopwatch	
Disposable absorbent material (e.g. tissue paper)	
For manual processing: microplate cover to protect reagent wells against evaporation	

Compatible products (not included in the test kit)

Description	EUROIMMUN order number
EUROIMMUN Analyzer I (software from version 1.96.3)	YG 0014-0101
EUROIMMUN Analyzer I-2 P (software from version 1.11)	YG 0015-0101
EUROLabWorkstation ELISA (software from version 2.5.2)	YG 0851-0101

Storage and stability

The test kit has to be stored at a temperature between +2 °C and +8 °C, do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

In-use stability

After initial opening, the product is stable until the indicated expiry date when stored at +2 °C to +8 °C and protected from contamination, unless stated otherwise below.

Warnings and precautions

- The product must only be used by healthcare professionals in suitable laboratory rooms.
- Do not use the product if the packaging of the reagents is damaged.
- Before using the product, read the instructions for use carefully. Only use the valid version, which can be downloaded from the customer portal (https://products.euroimmun.de).
- EUROIMMUN products must not be mixed with or replaced by products from other manufacturers.
- Wash buffer, enzyme conjugate, sample buffer, substrate and stop solution are exchangeable independent of the lots if they have identical article numbers (see labelling). All other reagents are lot-specific and must not be combined with other lots.

- Observe Good Laboratory Practice (GLP) and safety guidelines. Some of the reagents contain preservatives.
- The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all reagents should be treated as potentially infectious and be handled with care.
- Information on classification of the components according to CLP 1272/2008 is included in the safety data sheet. This is available in the customer portal (https://products.euroimmun.de/).

  The hazard and precautionary statements can be found in the following table.

Stop solution 0.5 M sulphuric acid, ready for use: ZE 1210-0112	
H/P code	**H/P phrase**
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves, protective clothing, eye protection, face protection.
P301+P330+P331+P310	IF SWALLOWED: rinse mouth. Do NOT induce vomiting. Immediately call a doctor, a POISON CENTER.
P303+P361+P353+P310	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a doctor, a POISON CENTER.
P305+P351+P338+P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER, a doctor.

Preparation and stability of samples

Sample material

Human serum or EDTA, heparin or citrate plasma.

Notes

- The use of products approved for the collection, storage and transport of serological samples ensures a sample quantity and quality suitable for the performance of the test.
- All preanalytical procedures must be performed according to the instructions of the manufacturers of the sample collection systems and to standard regulations, e.g. of the Clinical Laboratory Standards Institute (CLSI). Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline, 4th edition).
- Special pre-analytical characteristics, e.g. a predilution of the samples, are to be evaluated individually.

Sample preparation

The **patient samples** to be investigated are diluted **1:101** with sample buffer.
For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

Note

- The calibrators and controls are ready for use, do not dilute them.

Stability of the patient samples

- stored at +2 °C to +8 °C: up to 14 days
- stored at -25 °C to -15 °C: 4 freeze-thaw cycles

Preparation and stability of the reagents

Note

All reagents must be brought to room temperature (+18 °C to +25 °C). The reagents can be removed from the test kit to accelerate acclimatisation.

The thermostat adjusted ELISA incubator must be set at +37 °C ± 1 °C.

Sample buffer

Ready for use

Coated wells

Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2 °C and +8 °C for 4 months.

Calibrators and controls

Ready for use. The reagents must be mixed thoroughly before use.

Wash buffer

The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37 °C and mix well before diluting. The required volume must be diluted 1:10 with deionised or distilled water (1 part wash buffer plus 9 parts distilled water).
For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
The working-strength wash buffer is stable for 4 weeks when stored at +2 °C to +8 °C and handled properly.

Enzyme conjugate

Ready for use. The enzyme conjugate must be mixed thoroughly before use.

Chromogen/substrate solution

Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

Stop solution

Ready for use.

Waste disposal

Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All products must be disposed of in accordance with legal regulations.

Quality control

For every group of tests performed, the extinction values of the calibrators as well as the relative units and/or ratio values determined for the positive and the negative control must lie within the limits stated for the lot. The respective data can be found in the quality control certificate. If at least one of these calibrator and control requirements is not met, the test results must be considered invalid and the test must be repeated.

Metrological traceability

As no international reference material exists for the detection of IgG antibodies against TBE virus, the results are given as relative units that serve as a relative measurement of the antibody concentration in serum or plasma. The calibration was performed against in-house reference samples, which were used in the evaluation of the test system.

Test procedure

See quality control certificate for a schematic overview

For **semiquantative analysis** incubate **calibrator 3** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1 to 4** along with the positive and negative controls and patient samples.

Sample incubation: (1st step)

Transfer **100 µl of the calibrators, positive or negative control or diluted patient samples** into the individual microplate wells according to the pipetting protocol.
For manual processing protect the reagent wells with a suitable cover to prevent evaporation. For the automated test procedure, the instructions for use of the respective automated system must be followed.
Incubate **60 minutes at +37 °C ±1 °C**.

Washing:

Manual: Empty the wells and subsequently wash **3 times using 300 µl of working-strength wash buffer** for each wash.
Automatic: Wash the reagent wells **3 times with 450 µl of working-strength wash buffer** (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on disposable absorbent material with the openings facing downwards to remove all residual wash buffer.

Caution:
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette **100 µl of enzyme conjugate** (peroxidase-labelled antibody against human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18 °C to +25 °C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette **100 µl of chromogen/substrate solution** into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18 °C to +25 °C) (protect from direct sunlight).

Stopping:

Pipette **100 µl of stop solution** into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 3	P 6	P 14	P 22			C 1	P 3	P 11	P 19		
B	pos.	P 7	P 15	P 23			C 2	P 4	P 12	P 20		
C	neg.	P 8	P 16	P 24			C 3	P 5	P 13	P 21		
D	P 1	P 9	P 17				C 4	P 6	P 14	P 22		
E	P 2	P 10	P 18				pos.	P 7	P 15	P 23		
F	P 3	P 11	P 19				neg.	P 8	P 16	P 24		
G	P 4	P 12	P 20				P 1	P 9	P 17			
H	P 5	P 13	P 21				P 2	P 10	P 18			

The pipetting protocol for microplate strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).

The pipetting protocol for microplate strips 7 to 10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 4), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The reagent wells are in break-off format. This makes it possible to adjust the number of test substrates used to the number of samples to be examined, which minimises reagent wastage.

The positive and negative control are used for internal verification of the reliability of the test procedure. They must be assayed with each test run.

Test evaluation

Semiquantitative

The test can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 3. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 3}} = \text{Ratio}$$

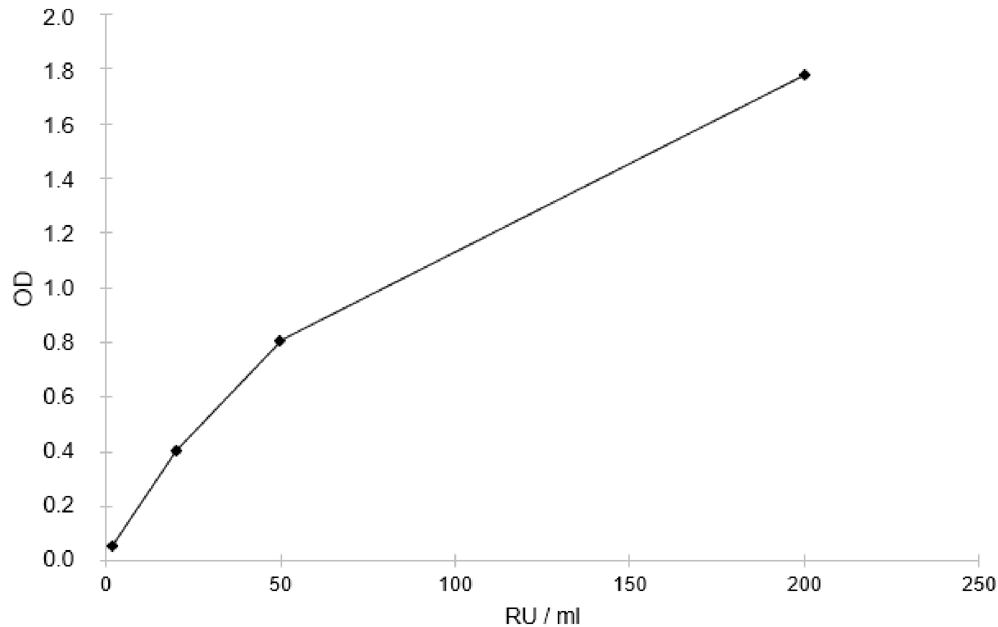
The cut-off recommended by EUROIMMUN is 1.0 ratio. EUROIMMUN recommends interpreting results as follows:

Ratio <0.8: negative
Ratio ≥0.8 to <1.1 borderline
Ratio ≥1.1 positive

For duplicate determinations the mean of the two values should be taken.

Quantitative

The standard curve from which the concentration of antibodies in the serum samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 4 calibrators against the corresponding units (linear/linear). Use the “point-to-point” evaluation method for the calculation of the standard curve. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above that of calibrator 1 (200 RU/ml), the result should be given as “>200 RU/ml”. It is recommended that this sample be remeasured in a new test run at a dilution of e.g. 1:400. The result in RU/ml read from the standard curve must then be multiplied by the respective dilution factor.
The upper limit of the normal range for non-infected persons (cut-off value) recommended by EUROIMMUN is 20 relative units (RU)/ml. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

Conversion of results into Vienna Units (VIEU/ml)

Vienna units were introduced in Vienna in 1983 by Hofmann et al. as a standard for the quantification of antibodies against TBE virus [9]. 29 samples from a TBE endemic area (origin: Austria) were incubated using the Anti-TBE Virus ELISA 2.0 (IgG) and the EUROIMMUN Anti-TBE Virus ELISA “Vienna” (IgG). From the results in relative units (RU/ml) or in Vienna units (VIEU/ml), a factor was determined to convert relative units into Vienna units. The factor applies specifically to the Anti-TBE Virus ELISA 2.0 (IgG). To convert test results given in RU/ml to VIEU/ml, the respective values must be multiplied by the factor **10.8**.

Analytical performance

Measurement range: 2 RU/ml – 200 RU/ml

Limit of blank (LoB): 2.12 RU/ml

Limit of detection (LoD): 2.63 RU/ml

Limit of quantitation (LoQ): 2.63 RU/ml

LoB, LoD and LoQ were determined according to the requirements defined in guideline EP17-A2 of the CLSI (Clinical and Laboratory Standards Institute, https://clsi.org/).

Linearity

Linearity of the Anti-TBE Virus ELISA 2.0 (IgG) is given in the range of 17 RU/ml to 189 RU/ml (with a maximum non-linearity of 20%). The linearity was determined according to the requirements defined in the CLSI guideline EP06-A.

Precision (repeatability and reproducibility)

Within-laboratory precision and between-lot precision studies were performed according to CLSI guideline EP05-A3. 6 samples (reactivity distributed over the entire measuring range) were measured. Precision is reported in terms of standard deviation (SD) and coefficient of variation (CV).

The **within-lab precision** is based on repeated measurements per test run (repeatability, within-run), from different test runs (between-run), on different days (between-day) and the resulting overall precision (within-lab).

The overall study design includes measurements on 20 days with 2 test runs and 2 replicates each (a total of 80 measurements per sample).

	Within-lab precision										
Sample	Mean value RU/ml	Repeatability, within-run		Between-run		Within-day		Between-day		Within-lab	
		SD	CV in %	SD	CV in %	SD	CV in %	SD	CV in %	SD	CV in %
1	4.91	0.280	5.7	0.402	8.2	0.490	10.0	0.167	3.4	0.517	10.5
2	15.90	0.440	2.8	0.570	3.6	0.720	4.5	0.281	1.8	0.773	4.9
3	24.43	0.647	2.6	1.128	4.6	1.300	5.3	1.101	4.5	1.704	7.0
4	19.35	0.379	2.0	0.951	4.9	1.024	5.3	0.898	4.6	1.362	7.0
5	76.33	1.815	2.4	3.001	3.9	3.508	4.6	5.912	7.7	6.874	9.0
6	130.92	2.741	2.1	4.324	3.3	5.120	3.9	3.949	3.0	6.465	4.9

The **between-lot precision** is based on repeated measurements per test run (repeatability, within-run), from different test runs (between-run), on different days (between-day) with different lots (between-lot) and the resulting overall precision (reproducibility).

The overall study design includes measurements of 3 lots, on 5 days with 2 test runs and 3 replicates each (a total of 90 measurements per sample).

In this study, the between-lot variability had no influence on the overall reproducibility compared to the within-lot and between-run variability. Therefore, the between-lot reproducibility is given with a CV of 0.0%.

	Between-lot precision												
Sample	Mean value RU/ml	Repeatability within-run		Between-run		Between-day		Within-lot		Between-lot		Reproduci-bility	
		SD	CV in %	SD	CV in %	SD	CV in %	SD	CV in %	SD	CV in %	SD	CV in %
1	5.10	0.225	4.4	0.282	5.5	0.244	4.8	0.436	8.6	0.000	0.0	0.436	8.6
2	15.92	0.737	4.6	0.227	1.4	0.355	2.2	0.849	5.3	0.000	0.0	0.849	5.3
3	22.83	0.865	3.8	0.926	4.1	0.264	1.2	1.294	5.7	0.000	0.0	1.294	5.7
4	18.14	0.370	2.0	0.623	3.4	0.334	1.8	0.797	4.4	0.000	0.0	0.797	4.4
5	68.05	1.880	2.8	3.050	4.5	0.000	0.0	3.583	5.3	0.000	0.0	3.583	5.3
6	124.87	4.033	3.2	3.008	2.4	2.402	1.9	5.575	4.5	0.000	0.0	5.575	4.5

Cross-reactivity (analytical specificity)

The quality of the antigen used ensures a high specificity of the ELISA. Possible serological cross reactivities within the flavivirus genus have been described in literature many times [10 – 15]. The cross reactivity was investigated by analysing samples positive for IgG antibodies against dengue, yellow fever, hepatitis C, Japanese encephalitis, West Nile and Zika virus using the Anti-TBE Virus ELISA 2.0 (IgG). The analysis showed that cross reactions with antibodies against yellow fever and hepatitis C virus are unlikely but that cross reactions with antibodies against dengue, Japanese encephalitis, West Nile and Zika virus are likely. It must be taken into account that double infections may occur, especially in endemic areas, or that an infection with another flavivirus may have taken place at an earlier point in time. In these cases, positive results are not caused by a cross reaction of the corresponding antibodies.

Antibodies against	**n**	**Positive rate in % in the Anti-TBE Virus ELISA 2.0 (IgG)**
Yellow fever virus	12	0.0
Dengue virus	27	85.2 (23 positive)
Hepatitis C virus	6	0.0
West Nile virus	30	50.0 (15 positive)
Zika virus	22	86.4 (19 positive)
Japanese encephalitis virus	12	58.3 (7 positive)

Interference

Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin in this ELISA.

Method comparison (accuracy)

28 precharacterised patient samples and 92 samples from apparently healthy blood donors (origin: Europe) were investigated using the Anti-TBE Virus ELISA 2.0 (IgG) from EUROIMMUN and a commercially available ELISA of another manufacturer as a reference. The positive agreement was 100% (95% CI: 91.4 - 100) and the negative agreement was 94.4% (95% CI: 86.4 - 98.5) with a mean concordance of 96.5%. Results in the borderline range were not included in the calculation.

n = 120		**ELISA of another manufacturer**			
		positive	borderline	negative	total
Anti-TBE Virus ELISA 2.0 (IgG), EUROIMMUN	positive	41	7	4	52
	borderline	0	0	0	0
	negative	0	0	68	68
	total	41	7	72	120

Clinical performance

A total of 271 samples were tested using the Anti-TBE Virus ELISA 2.0 (IgG). For the determination of sensitivity, samples from patients with acute and past TBEV infection as well as samples from persons vaccinated against TBEV were used. For the determination of specificity, samples from healthy blood donors as well as samples from patients with the relevant differential diagnosis anaplasmosis, herpes simplex virus infection and SARS-CoV-2 infection were investigated.

The results are shown in a contingency table below:

		Clinical characterisation		
		Positive	Negative	Total
EUROIMMUN test system	Positive	144	5	149
	Borderline	1	0	1
Anti-TBE Virus ELISA 2.0 (IgG)	Negative	0	121	121
	Total	145	126	271

Excluding borderline results, the performance parameters are as follows:

Evaluation	n = 270	
	%	**95 %-CI**
Specificity	96.0	91.0% - 98.7
Sensitivity	100.0	97.5% - 100.0

Positive likelihood ratio: >10

Negative likelihood ratio: <0.1

Prevalence

Levels of anti-TBE virus antibodies were determined in a panel of healthy blood donors (n = 500, origin: Schleswig-Holstein, Germany, year 2022) using this ELISA. With a cut-off of 20 RU/ml, 23.6% of the blood donors were positive for anti-TBE virus (IgG). This result lies within the expected range for the prevalence for this region in Germany. In a panel of healthy blood donors (n = 500, origin: Schleswig-Holstein, Germany) from 2012, the positive rate was still 14.8%. This development corresponds to the expected increase of the prevalence of anti-TBE virus IgG antibodies in the German population over time.

Limitations of the procedure

- The results should always be interpreted together with those of further laboratory diagnostic procedures and based on the clinical picture.
- The specifications in these instructions for use, e.g. pipetting volumes, incubation times, temperatures, preparation steps, as well as the specifications for preanalytics must be observed to avoid incorrect results.
- Partial or complete adaptation of the test system for use with automated sample processors or other liquid handling devices may lead to differences between the results obtained with the automated and manual procedure. It is the responsibility of the user to validate the automated instruments used for the analysis to ensure that they yield test results within the permissible range.
- Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes or too short reaction times) can lead to falsely extinction values.
- Residual liquid (>10 µl) remaining in the reagent wells after washing can interfere in the substrate conversion and lead to false low extinction values.
- Correct sample collection and storage are crucial for the reliability of the results.

- The binding activity of the antibodies and the activity of the enzyme used are temperature dependent. It is therefore recommended to use a thermostatically controlled ELISA incubator in all incubation steps.

- A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In the case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative or borderline serological result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. A significant increase in the specific IgG antibody level (by more than twofold) and/or seroconversion in a follow-up sample taken after at least 7 to 10 days may be interpreted as an indication of acute infection. The sample and the follow-up sample should be incubated in the same test run.

Literature

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Liability

The test kit, including original accessories, must only be used in accordance with the intended use. EUROIMMUN accepts no liability for any other use (e.g. non-compliance with the instructions for use and improper use) or for resulting damages.

Technical support

In case of technical problems, please contact us via our website (https://www.euroimmun.de/en/contact/). Instructions for use, test kit information, certificates and safety data sheets are available in the customer portal (https://products.euroimmun.de/). They can also be ordered by telephone on +49 451 2032 0.

Additional information

- Further information on the product can be found in the European Database on Medical Devices (EUDAMED).
- Regulatory information for customers in the European Union: Please observe the obligation to report any serious incidents occurring in connection with this product to the competent authorities and to EUROIMMUN.

Meaning of the symbols

The following symbols are used on the packaging and in the instructions for use.

Symbol	Meaning	Symbol	Meaning
STRIPS	Microplate strips	LOT	Batch code
CAL 1	Calibrator 1	UDI	Unique Device Identifier
CAL 2	Calibrator 2	CE 0197	CE marking with ID number of TÜV Rheinland LGA Products GmbH
CAL 3	Calibrator 3		Keep away from sunlight
CAL 4	Calibrator 4		Temperature limit
POS CONTROL	Positive control		Use by date (YYYY-MM-DD)
NEG CONTROL	Negative control		Date of manufacture (YYYY-MM-DD)
CONJUGATE	Enzyme conjugate		Manufacturer
SAMPLE BUFFER	Sample buffer		Consult instructions for use
WASH BUFFER 10x	Wash buffer, 10x concentrate	REF	Catalogue number
SUBSTRATE	Chromogen/substrate solution	Σ	Contains sufficient for <n> tests
STOP SOLUTION	Stop solution		Biological risks
QC CERTIFICATE	Quality control certificate	IVD	In vitro diagnostic medical device
BIO	Contains biological material of animal origin	BIO	Contains biological material of human origin

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