Anti-Zika Virus ELISA (IgG)

- **Worldwide first specific serological test**
- **No cross reactions due to the use of virus-specific NS1 antigen**
- **Discrimination from other symptomatically similar viral infections (e.g. dengue or chikungunya)**

Technical data

Antigen	Recombinant non-structural protein (NS1) of Zika virus
Calibration	Quantitative, in relative units per millilitre (RU/ml) Calibration serum 1: 200 RU/ml Calibration serum 2: 20 RU/ml Calibration serum 3: 2 RU/ml Recommended upper threshold of the reference range for non-infected individuals (cut-off): 20 RU/ml
Sample dilution	Serum or plasma, 1:101 in sample buffer
Reagents	Ready for use, with the exception of the wash buffer (10x); colour-coded solutions, in most cases exchangeable with those in other EUROIMMUN ELISA kits
Test procedure	60 min (37 °C) / 30 min / 15 min, room temperature, fully automatable
Measurement	450 nm, reference wavelength between 620 nm and 650 nm
Test kit format	96 break-off wells; kit includes all necessary reagents
Order number	**EI 2668-9601 G**

Clinical significance

Zika virus belongs to the arboviruses of the Flaviviridae family. The virus is generally not transmitted between humans. In individual cases, however, transmission via sexual intercourse has been reported. The virus is transmitted through bites of mosquitoes of the genus Aedes. Non-human primates serve as the virus reservoir. The virus was first observed in African countries. In recent years there have been major outbreaks in tropical and subtropical regions in Asia and on Pacific islands. Recently an increasing number of infections has been observed in Brazil. In most cases the disease course is mild. The symptoms are near-to identical to those of dengue or chikungunya virus infection. After an incubation time of five to ten days a flu-like illness develops with fever, rash, arthralgia, myalgia, headache and conjunctivitis. A connection between Zika virus infection in pregnancy and microcephaly in the newborn, and the occurrence of neurological diseases such as Guillain Barré syndrome (GBS) after Zika virus infection are currently being discussed. There is no specific treatment for ZIKV infection. Protection from mosquito bites serves as a preventative measure. Vaccination is not yet available. Infections with flaviviruses, including ZIKV, always lead to viraemia. Since the detection of the virus or its components is only possible during the viraemic phase (up to one week) and the relatively mild disease course often causes patients to seek medical advice at an advanced stage of disease, serological investigation is of major importance. Specific antibodies can be detected several days after the onset of symptoms.

Diagnostic application

The Anti-Zika Virus ELISA (IgG, IgM) is suitable for the serodiagnosis of acute and past Zika virus infections. Due to the use of virus-specific NS1 antigen, cross reactions can be virtually excluded. Thus, Zika virus infections can be discriminated from infections with other viruses such as dengue and chikungunya, which cause similar symptoms and are endemic in the same regions. The detection of virus-specific IgM antibodies or a significant increase in the IgG titer in a follow-up sample indicates an acute infection. Further, the determination of specific antibodies is relevant for epidemiological studies and for clarification of possible links between Zika virus infection and other diseases (GBS, microcephaly).

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Detection limit

The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest clearly detectable antibody titer. The lower detection limit of the Anti-Zika Virus ELISA (IgG) is 0.44 RU/ml.

Reference range

The levels of anti-Zika virus antibodies (IgG) were analysed with the EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off value of 20 IU/ml, 0.2 % of the blood donors were anti-Zika virus positive (IgG).

Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 6 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on two determinations performed in ten different test runs.

	Intra-assay variation, n = 20		Inter-assay variation, n = 2 x 10	
Serum	Mean value (RU/ml)	CV (%)	Mean value (RU/ml)	CV (%)
1	5.6	5.7	6.3	11.6
2	11.3	3.5	12.3	5.6
3	12.1	3.2	13.7	4.7
4	13.7	3.5	15.8	5.5
5	47.6	6.2	51.5	10.6
6	73.9	5.4	74.1	9.7

Specificity and sensitivity

129 samples were investigated with the Anti-Zika Virus ELISA (IgG) and the Anti-Zika Virus ELISA (IgM). 29 samples originated from patients who had tested positive for Zika virus in examinations of the WHOCC. Serological and clinical data are available. The reference group comprised 100 samples from healthy pregnant women without Zika virus contact. The sensitivity of the Anti-Zika Virus ELISA for both immunoglobulin classes together was 97 %. The specificity of each assay was 100 %. When considering the immunoglobulin classes separately, the sensitivity was 76 % for the Anti-Zika Virus ELISA (IgG) and 86 % for the Anti-Zika Virus ELISA (IgM).

n = 129		WHOCC / Routine laboratory		
		positive	borderline	negative
EUROIMMUN Anti-Zika Virus ELISA (IgG and IgM) together	positive	28	0	0
	borderline	1*	0	0
	negative	0	0	100

*Sample showed a borderline anti-Zika virus IgM result

Origin of samples: 29 patient samples with Zika virus infection: WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research (WHOCC), Hamburg, Germany; 100 samples from healthy pregnant women: Routine laboratory, Germany

For evaluation of the specificity of the Anti-Zika Virus ELISA (IgG) a study was performed on 72 patient samples that were seropositive for rheumatoid factors and a variety of other autoantibodies (ANA). 22 additional samples originated from patients with acute EBV infection. Of the 94 samples in total all sera were found to be negative with the Anti-Zika Virus ELISA (IgG).

Possible influencing factors	n	Anti-Zika Virus ELISA (IgG) positive
Acute EBV infection	22	0 %
Various autoantibodies (ANA)	35	0 %
Rheumatoid factors	37	0 %

Cross reactivity

By the use of a highly specific recombinant protein cross reactions are virtually prevented. Serum panels from clinically and serologically well characterised patients with high antibody titers of classes IgG and/or IgM against other flaviviruses and chikungunya virus were investigated. Only one patient with acute JEV infection showed a positive result with the Anti-Zika Virus ELISA (IgG).

Note: Double infection or infection with another flavivirus at an earlier time are possible, particularly in endemic areas. In this case, positive results are not caused by a cross reactivity of the corresponding antibodies.

Antibodies against	n	Anti-Zika Virus ELISA (IgG) positive
Chikungunya virus	19	0 %
Dengue virus	38	0 %
TBE virus	15	0 %
Yellow fever virus	12	0 %
Japanese encephalitis virus (JEV)	25	4 %
West Nile virus	34	0 %

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