Anti-Zika Virus ELISA (IgG)

- **First specific serological test worldwide for the detection of antibodies against Zika virus for acute and past Zika virus infections**
- **Cross reactions are virtually excluded 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 temp. (sample / conjugate / substrate incubation), 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 (ZIKV) is an arbovirus 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. 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 Latin America. In most cases the disease course is mild. The symptoms are near-to identical to those of dengue or chikungunya virus infections. After an incubation time of five to ten days a flu-like illness develops with fever, rash, arthralgia, myalgia, headache and conjunctivitis. During the Zika outbreaks in Polynesia 2014 and Latin America 2015/16, a significant increase in neurological diseases such as Guillain-Barré syndrome was registered. Especially in Brazil, an exceptionally high number of babies were born with microcephaly, a cranial-cephalic malformation. The connection between the presence of a ZIKV infection and the occurrence of neurological diseases or foetal malformations in considered as virtually proven. There is no specific treatment for ZIKV infection. Protection from mosquito bites serves as a preventative measure. Since the detection of the virus or its components is only possible during the viraemic phase (up to one week after infection) 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. The detection of virus-specific IgA and/or IgM antibodies indicate an acute infection. IgG antibody detection should be performed as a supplement to the specific IgA and/or IgM antibody detection. Seroconversion or a significant increase in the IgG antibody titer indicates an acute infection.

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Reference range

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

Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using six 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	
Sample	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

Sensitivity and specificity

A study was performed on 38 patients from ZIKV-endemic regions (origin: Dominican Republic, Colombia) and 33 samples from European returning travellers whose first samples was classified as positive using the ZIKV RT-PCR. The serological investigation was performed on samples withdrawn >5 days after onset of symptoms. 33 samples from patients in which a dengue virus infection was confirmed at the first withdrawal were used as a control panel (returning travellers, origin: Germany). Due to the positive precharacterisation, the follow-up samples were used to determine the specificity. The sensitivity of the ELISAs, taking into consideration both immunoglobulin classes (IgG and IgM), amounted to 100% (IgG 100%, IgM 56%) at a specificity of 94% (IgG and IgM, each 97%), The sensitivity of the Anti-Zika Virus ELISA (IgM) amounted to 27% in patient from ZIKV endemic regions and to 87% in European returning travellers.

n = 104		**Confirmed ZIKV infection** (RT-PCR positive)/ **control panel** (confirmed dengue virus infection)		
		positive	borderline	negative
EUROIMMUN Anti-Zika Virus ELISA (IgG and IgM) together	positive	71	0	2
	borderline	0	0	0
	negative	0	0	31

It must be taken into account that patients who had previously had contact with a Flavivirus (infection or vaccination) and have now again had contact with a representative of this virus genus (secondary flavivirus infection) may only form a small or even undetectable amount of specific IgM antibodies. In this case, specific IgM antibodies are not detected despite the fact that there is an acute infection.

To determine the specificity of the Anti-Zika Virus ELISA (IgG), a further study was performed with 72 sera which were seropositive for rheumatoid factors, and various autoantibodies (ANA). 22 additional samples originated from patients with acute EBV infection. In this special panel, the specificity amounted to 100%.

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

The recombinant NS1 protein of ZIKV has proven to be a highly specific target structure for the immune response in internal and external studies. Despite significant homologies within the flavivirus genus, cross reactions can be virtually excluded when using the NS1 protein. In order to investigate the cross reactivity, sera from patients with dengue virus infection which showed high antibody titers of classes IgA and IgG and/or IgM, from patients after TBE or yellow fever vaccination, and from patients with JEV or WNV infections were analysed. Only two patients with acute JEV or DENV infection showed a positive result with the Anti-Zika Virus ELISA (IgG).

Antibodies against	**n**	**Anti-Zika Virus ELISA (IgG) positive**
Dengue virus (DENV)	146	1%
TBE virus (TBEV)	153	0%
Yellow fever virus (YFV)	12	0%
Japanese encephalitis virus (JEV)	25	4.0%
West Nile virus (WNV)	74	0%

Note: It must be taken into account that double infections may occur, especially in endemic regions, and there may have been an infection with another flavivirus at an earlier time point. In this case, positive results are not caused by a cross reactivity of the corresponding antibodies. Since interference cannot be excluded in samples from patients with acute Plasmodium spp. infections, malaria should always be taken into account in differential diagnoses.

Literature

Steinhagen K, et al. **Serodiagnosis of Zika virus infections by a novel NS1-based ELISA devoid of cross-reactivity with dengue virus antibodies.** A multicohort study of assay performance, 2015 to 2016. Euro Surveill 21(50):pii=30426 (2016).

Huzly, et al. **High specificity of a novel Zika virus ELISA in European patients after exposure to different flaviviruses.** Euro Surveill 21(16):pii=30203 (2016).

Granger, et al. **Zika virus antibody detection: evaluation of three different serologic methodologies**. Poster at CVS 2016 (USA).

Borena et al. **No molecular or serological evidence of Zikavirus infection among healthy blood donors living in or travelling to regions where Aedes albopictus circulates.** PLoS One 12(5):e0178175 (2017).

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