Anti-Zika Virus ELISA (IgA)

- **First specific serological test worldwide for the detection of antibodies against Zika virus**
- **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	Semiquantitative; calculation of a ratio from the extinction values of the control or sample and the extinction values of the calibrator
Result interpretation	EUROIMMUN recommends interpreting results as follows: Ratio <0.8: negative Ratio ≥0.8 to <1.1: borderline Ratio ≥1.1: positive
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 A**

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. ZIKV 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 DENV 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 2015/2016, 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 is 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.

Diagnostic application

The Anti-Zika Virus ELISA (IgA, IgG, IgM) is suitable for the serodiagnosis of acute and past ZIKV infections. Due to the use of virus-specific NS1 antigen, cross reactions can be virtually excluded. Thus, ZIKV 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 IgA and/or IgM antibodies indicates 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. Furthermore, the determination of specific antibodies is relevant for epidemiological studies and for clarification of possible links between ZIKV infection and other diseases.

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Reference range

The levels of anti-ZIKV antibodies (IgA) were analysed with the EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of ratio 1.0, none of the blood donors was anti-ZIKV positive (IgA).

Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using four 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 (ratio)	CV (%)	Mean value (ratio)	CV (%)
1	0.5	7.8	0.2	10.9
2	0.9	3.6	1.2	8.4
3	2.3	3.8	3.0	10.4
4	2.3	3.8	7.9	10.3

Sensitivity and specificity

A study was performed on 38 patients from ZIKV-endemic regions (origin: Dominican Republic, Colombia) and 11 samples from European returning travellers, whose first samples had been classified as positive by ZIKV RT-PCR. The serological investigation was performed in samples withdrawn >5 days after onset of symptoms. 33 patient samples, in which a dengue virus infection could be 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 EUROIMMUN Anti-Zika Virus ELISA (IgA) was 92 %, with a specificity of 97 %.

n = 82		**Confirmed ZIKV infection** (RT-PCR positive) / **control panel** (confirmed dengue virus infection)		
		positive	borderline	negative
EUROIMMUN Anti-Zika Virus ELISA (IgA)	positive	45	0	1
	borderline	0	0	0
	negative	4	0	32

For evaluation of the specificity of the Anti-Zika Virus ELISA (IgA) a study was performed on 71 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. In this special panel, the specificity amounted to 100 %.

Possible influencing factors	n	Anti-Zika Virus ELISA (IgA) positive
Acute EBV infection	22	0 %
Various autoantibodies (ANA)	35	0 %
Rheumatoid factors	36	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 the significant homologies within the Flavivirus genus, cross reactions can virtually be excluded when using the NS1 protein. In order to investigate the cross reactivity, sera from patients with dengue virus infections, which showed high antibody titers of classes IgA and IgG and/or IgM, from patients after TBE or yellow fever vaccinations, and from patients with JEV or WNV infections were analysed. Only two patients from this panel showed a positive reaction with the Anti-Zika Virus ELISA (IgA).

Antibodies against	n	Anti-Zika Virus ELISA (IgA) positive
Dengue virus (DENV)	96	1.0 %
TBE virus (TBEV)	69	0 %
Yellow fever virus (YFV)	12	0 %
Japanese encephalitis virus (JEV)	13	0 %
West Nile virus (WNV)	56	1.8 %

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 diagnosis.

Literature

1. Steinhagen 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. 2016;21(50):pii=30426.
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3. Barzon L, Percivalle E, Pacenti M, Rovida F, Zavattoni M, Del Bravo P, Cattelan AM, Palù G, Baldanti F. Virus and Antibody Dynamics in Travelers With Acute Zika Virus Infection. Clin Infect Dis. 2017 Dec 30. doi: 10.1093/cid/cix967.

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