Anti-Zika Virus ELISA (IgAM)

- **Combined detection of IgA and IgM antibodies against Zika virus to support diagnosis of acute 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	Semiquantitative; calculation of a ratio from the extinction of the sample and the extinction 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 Q**
Related products	EI 2668-9601 A, G or M: Anti-Zika Virus ELISA (IgA, IgG or IgM)

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 2014 in Polynesia and 2015/2016 in Latin America, 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 link 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 EUROIMMUN Anti-Zika Virus ELISA (IgAM) is a screening test for the serodiagnosis of acute 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. To support the specific IgAM detection, IgG should also be determined. Seroconversion or a significant increase in the IgG antibody titer also indicates an acute infection.

EUROIMMUN AG · Seekamp 31 · 23560 Lübeck (Germany) · Tel +49 451/5855-0 · Fax 5855-591 · info@euroimmun.de · www.euroimmun.com

Reference range

The levels of anti-ZIKV antibodies (IgAM) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors (origin: Germany). With a cut-off of ratio 1.0, no anti-ZIKV antibodies (IgAM) were detected.

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 14 different test runs.

	Intra-assay variation, n=20		Inter-assay variation, n=2 x 14	
Sample	Mean value (ratio)	CV (%)	Mean value (ratio)	CV (%)
1	0.2	3.1	0.3	7.8
2	0.3	2.0	0.4	6.0
3	0.9	6.0	1.0	6.5
4	0.9	5.5	1.1	6.2
5	2.3	2.5	1.2	7.0
6	2.7	5.5	1.5	5.0

Sensitivity and specificity

Samples from 31 patients from ZIKV endemic regions (origin: Dominican Republic), whose first samples were classified as positive by ZIKV RT-PCR, were investigated using the EUROIMMUN Anti-Zika Virus ELISA (IgAM). The serological investigation was performed on samples taken > 5 days after symptom onset. The control panel consisted of 24 samples from patients (returning travellers, origin: Germany) with dengue virus infections which were secured by direct detection on first samples. On the basis of the positive precharacterisation, the follow-up samples were used to determine the specificity. The sensitivity of the EUROIMMUN ELISA amounted to 100 % at a specificity of 91.3 % (excluding borderline sera).

n = 55		**Secured infection ZIKV** (RT-PCR positive) / **Control panel** (secured infection with dengue virus)		
		positive	borderline	negative
EUROIMMUN Anti-Zika Virus ELISA (IgAM)	positive	31	0	2
	borderline	0	0	1
	negative	0	0	21

For evaluation of the specificity of the EUROIMMUN Anti-Zika Virus ELISA (IgAM) a study was performed on 74 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 96.8 %.

Possible influencing factors	**n**	**Anti-Zika Virus ELISA (IgAM) positive**
Rheumatoid factors	33	3.0 %
Diverse autoantibodies (ANA)	41	0 %
Acute EBV infection	22	9.1 %

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. For investigation of the cross reactivity, sera from patients with dengue virus infections, from patients after TBE or yellow fever vaccination and from patients with HCV or WNV infections were analysed. Only one patient from the panel showed a positive result with the Anti-Zika Virus ELISA (IgAM).

Antibodies against	**n**	**Anti-Zika Virus ELISA (IgAM) positive**
Dengue virus (DENV)	27	0 %
TBE virus (TBEV)	25	0 %
Yellow fever virus (YFV)	12	0 %
Hepatitis C virus (HCV)	6	0 %
West Nile virus (WNV)	40	2.5 %

Note: It must be taken into account that double infections are possible, especially in endemic regions, or that an infection with another flavivirus may have occurred at an earlier time point. In this case, positive results are not caused by a cross reactivity of the corresponding antibodies. Since interference in samples with acute Plasmodium spp. infections cannot be excluded, 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.
2. Steinhagen K, Wilhelm N, Schmidt-Chanasit J, Emmerich P, Huzly D, Lattwein E, Fraune J., Schlumberger W, Warnecke J. Anti-Zika virus IgA may indicate an acute infection in anti-Zika virus IgM-negative patients. Scientific presentation at the 1st International Conference on Zika Virus, Washington DC, USA, February 2017.
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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