EUROIMMUN Medizinische Labordiagnostika AG

Zika virus infections

EUROIMMUN test systems for the diagnosis of Zika virus infections

Zika virus

The Zika virus (ZIKV) belongs to the family of flaviviruses which encompasses more than 50 different viruses. Dengue, yellow fever and West Nile viruses are the most prominent flaviviruses. Zika virus was first isolated in 1947, but only became more known due to a series of epidemics in recent years.

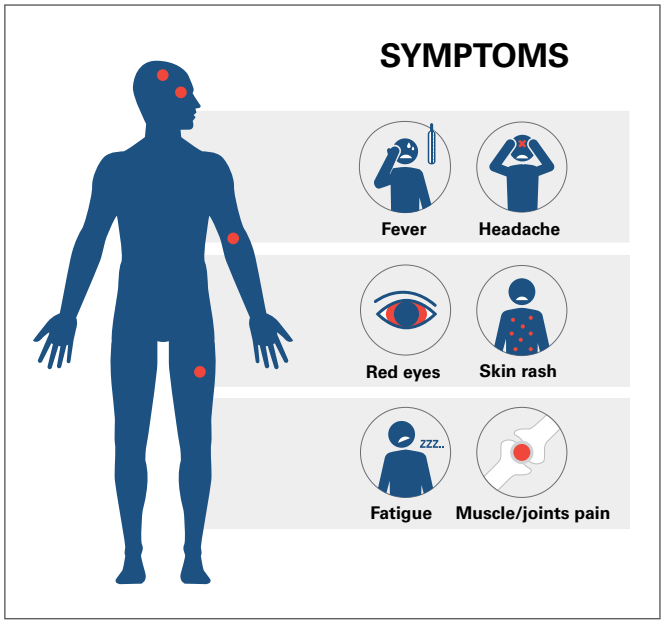
Since 2013, an increasing number of Zika virus outbreaks in different regions has been registered, for example in Southeast Asia, Polynesia and the Pacific region, some islands in the Caribbean, and in over 30 countries and regions in North, Central and South America.

The virus is transmitted to humans by the bite of mosquitoes of the Aedes genus. Infected female mosquitoes transmit the virus whilst feeding on blood. Another mode of transmission is perinatal transmission, i.e. transmission from an infected mother to the unborn child. Transmission through sexual intercourse and blood transfusions have also been reported from different countries.

Symptoms

Zika virus infections proceed asymptomatically in most cases (approx. 80 %). Only in 20 % of cases do patients show symptoms which usually encompass rash, fever, headache, pains in the joints, and conjunctivitis. These symptoms usually occur 3 to 12 days after the mosquito bite and persist for 2 to 7 days. The disease course is usually mild and self-limiting. The symptoms are very similar to those of dengue, or chikungunya virus infections.

In Brazil and several other countries, a significant increase in neurological disorders such as Guillain-Barré syndrome was registered during the Zika epidemic 2015/2016. Moreover, there was an exceptionally high number of babies with microcephaly. The relation between Zika virus infection and the occurrence of neurological disorders and foetal malformations is considered as virtually proven. The topic is currently intensively investigated.



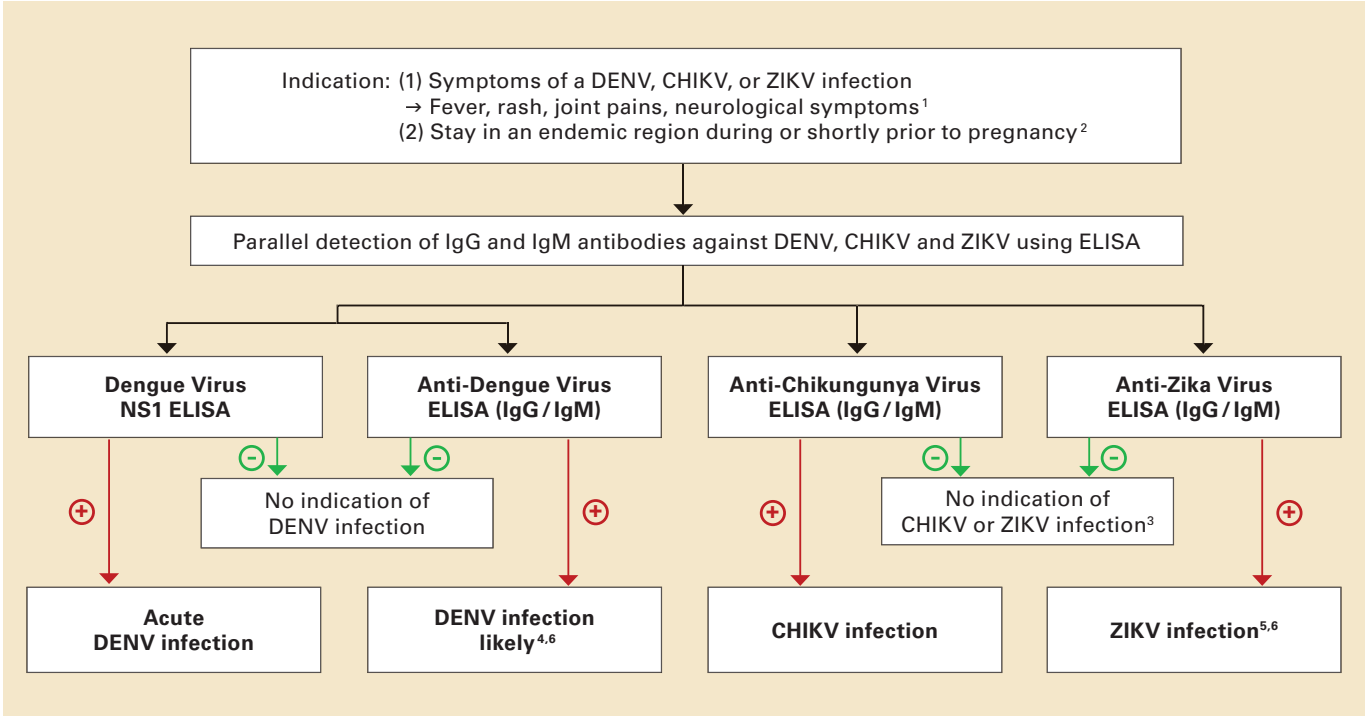
Diagnosis

Diagnosis of Zika virus infections can be made by direct detection of viral RNA or by indirect detection, i.e. determination of antibodies. The direct detection of the virus is possible for maximum one week after onset of symptoms. Specific antibodies are detectable for a longer period and can indicate both past and acute infections.

From approximately day 5 onwards, IgM antibodies can be detected in the serum of infected patients. Approximately 2 to 3 days after the appearance of IgM antibodies, also IgG antibodies are detectable. Both antibody classes can also appear at the same time. Antibodies can be detected by means of serological tests such as ELISA or indirect immunofluorescence (IIFT). In the interpretation of serological results, the structural similarity of flaviviruses must be taken into account, as this can cause cross reactions of the specific antibodies. In patients without past contact with other flaviviruses the cross reactivity is only minimal. In cases of prior infection or vaccination to another flavivirus, however, a significant cross reactivity must be expected. This can be virtually excluded by the use of highly specific NS1 antigen.

Serological differential diagnosis with ELISA test systems

In suspected cases of dengue, chikungunya or Zika virus (DENV, CHIKV, ZIKV) infection, EUROIMMUN recommends differential diagnosis following the procedure as described in the schemes below. If the blood sample was taken within 7 days after symptom onset, it is recommended to perform an additional RT-PCR test on serum or urine for direct pathogen detection.



[1] e.g. loss of mobility, numbness in the limbs, ascending pareses, facial pareses or loss of muscular reflex as a sign of Guillain-Barré syndrome (GBS).

[2] Men who have been to endemic regions and whose partner is pregnant should also be examined, since sexual transmission of Zika virus is possible.

[3] In clinically-supported suspected cases and in diagnosis during pregnancy, a follow-up sample should be taken within 1 to 2 weeks: If this is also negative, an acute infection is highly unlikely.

[4] Cross reactivity with other flaviviruses cannot be excluded. In secondary infections with other flaviviruses, the DENV IgG titer can be above the titer of the virus which causes the acute infection.

[5] False positive results can occur in sera from patients with acute plasmodium infections.

[6] Possible serological constellations and their relevance in flavivirus infections (e.g. DENV, ZIKV, tick-borne encephalitis virus, yellow fever virus, West Nile virus, etc.):

IgM	IgG	IgG titer increase in follow-up sample after 1–2 weeks	Indication of
+	–/+*	yes	**Acute infection** without prior contact with flavivirus **(primary infection)**
–/+**	+	yes	**Acute infection** after prior contact with flavivirus **(secondary infection)**
–	+	no	**Past infection** or previous virus contact

*IgG antibodies usually occur in parallel with IgM antibodies or shortly after. ** In cases of previous contact with other flavivirus, the IgM response can occur with a time delay, with reduced intensity, or not at all.

Study data of ELISA test systems

Sensitivity and specificity

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. 100 serum samples of healthy pregnant women were used as reference group. Sensitivity of the Anti-Zika Virus ELISA amounted to 97 %, and specificity to 100 % (taking into account both immunoglobulin classes). 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). Depending on the disease stage, it is possible that only one Ig class is present.

In another study, reference groups from different non-endemic regions were investigated. The panel encompassed sera from clinically inconspicuous blood donors (sample origin: Germany, USA, Africa and South America), healthy pregnant women (sample origin: Germany and USA) and healthy children (sample origin: Germany). The determined specificity of the IgG ELISA amounted to 99.8 %, and of the IgM ELISA amounted to 99.7 %.

The high specificity and low cross reactivity of the EUROIMMUN ELISA was confirmed in a study of the University Clinic Freiburg and the Bernhard-Nocht Institute for Tropical Medicine (Huzly et al., 2016). Serum samples from European patients with flavivirus infections or vaccinations, and samples from patients with acute virus infections were investigated. The study confirmed a specificity of 100 % for the Anti-Zika Virus ELISA (IgG) and 98 % specificity for the Anti-Zika Virus ELISA (IgM).

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

*29 samples from patients 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

Total number of sera: 1072	n	Anti-Zika Virus ELISA positive	
		IgG	IgM
Blood donors, Germany	500	0.2 %	0.2 %
Blood donors, USA	100	1 %	0 %
Blood donors, Florida, USA	57	0 %	1.7 %
Blood donors, Argentina	99	0 %	1 %
Blood donors, Zimbabwe	128	0 %	0 %
Pregnant women, Germany	100	0 %	0 %
Children ≤ 10 years, Germany	88	0 %	0 %

Cohort (Huzly et al.)	Anti-Zika Virus ELISA positive			
	n (IgG)	IgG	n (IgM)	IgM
TBE virus infection	21	0 %	38	0 %
Dengue virus infection	10	0 %	16	0 %
Yellow fever vaccine	15	0 %	15	0 %
Hepatitis C virus infection	16	0 %	–	–
Polyclonal IgM	–	–	52	5.8 %

Cross reactivity

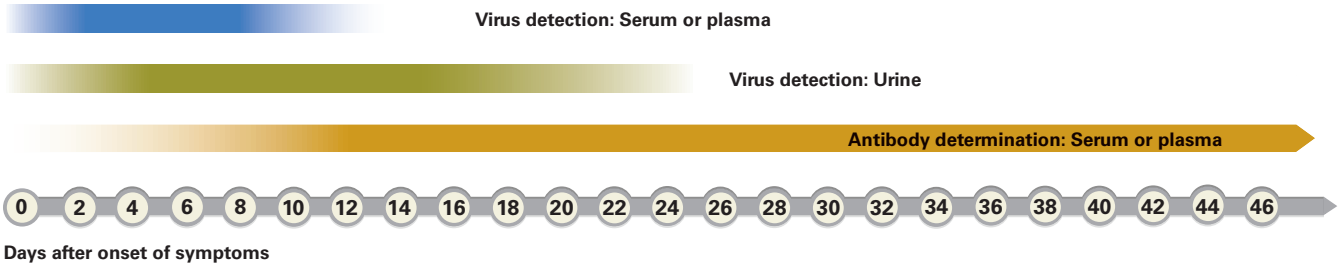
Due to the use of a highly specific, recombinant protein as antigen, cross reactions are virtually ruled out in the EUROIMMUN ELISAs. The investigation of sera panels of clinically and serologically characterised patients with high antibody titers of class IgG and IgM against other flaviviruses, chikungunya virus and Plasmodium species showed a very reduced cross reactivity. The dengue samples used originate from patients with confirmed dengue infections as determined by positive PCR and / or positive NS1 determination. The samples from patients with chikungunya originate from the CHIKV outbreak in La Réunion. Serological investigation of these samples from symptomatic patients with showed high antibody titers of the classes IgG and IgM against CHIKV.

Antibodies against	n	Anti-Zika Virus ELISA positive	
		IgG	IgM
Chikungunya virus	19	0 %	0 %
Dengue virus	119	0 %	0 %
Japanese encephalitis virus	25	4 %	0 %
Yellow fever virus	12	0 %	0 %
West Nile virus	34	0 %	2.9 %
TBE virus	81	0 %	0 %

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.

Time window for reliable diagnosis of Zika virus infections

The most suitable method for the detection of Zika virus infection depends on the disease stage. In the early phase, viral RNA can be determined. The Zika virus can be detected in blood by RT-PCR up to one week after onset of symptoms. In infected pregnant women, the virus can be detected also several weeks after this (according to Driggers et al., (2016)). Virus detection in urine by PCR is possible for a longer period of time than in serum or plasma. Here, positive results may occur up to 2 to 4 weeks after onset of symptoms. If the infection is older than 7 days, serological testing is recommended. Antibodies can be detected in the blood of the patient from day 5.



Days after onset of symptoms	Virus detection	Virus detection	Serology (IgG / IgM)
	RT-PCR serum	RT-PCR urine	ELISA or IIFT
1–7	+	+	–/+
8–27	–	+	+
from 28	–	–	+

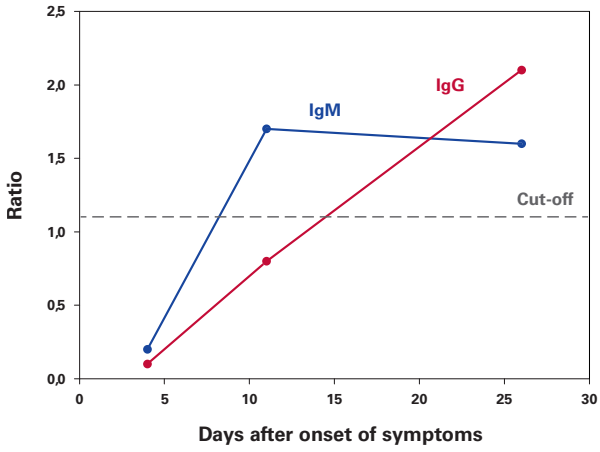
Case study PCR-positive patient

The Anti-Zika Virus ELISA (IgG and IgM) was evaluated in an external study. The sera panel encompassed 23 PCR-positive samples (total: 82 samples, including follow-up samples). In all patients, seroconversion could be observed, which shows a 100 % sensitivity. A typical case is shown below.

Patient returning from Brazil

- No antibodies detected in the first serum sample, withdrawn at day 4 after onset of symptoms. In this phase, PCR diagnostics are indispensable.
- In a follow-up sample, withdrawn at day 11, antibodies of class IgM are positive. Later, also antibodies of class IgG show positive.

Days after onset of symptoms	EUROIMMUN Anti-Zika Virus ELISA	
	IgM (ratio)	IgG (ratio)
4	0.2	0.1
11	1.7	0.8
26	1.6	2.1



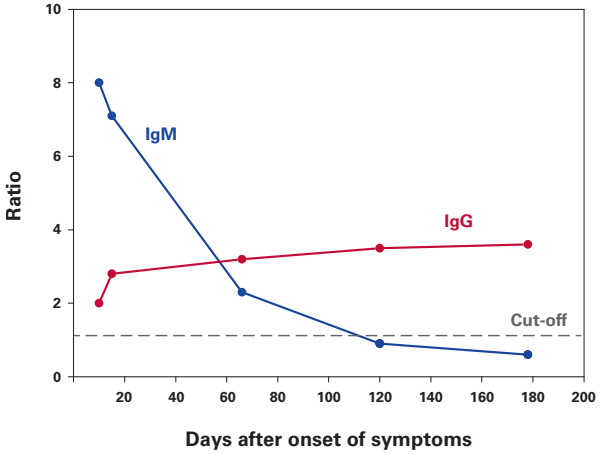
Semiquantitative test evaluation by ratio: Negative: Ratio < 0.8 Borderline: Ratio ≥ 0.8 to 1.1 Positive: Ratio ≥ 1.1

Case study: Returning from travel

Patient: Returning from travel to Colombia

- IgG level positive after onset of symptoms. Slow titer increase over a long period of time.
- IgM level firstly positive, decreasing in the further progression, and negative after approx. 120 days.

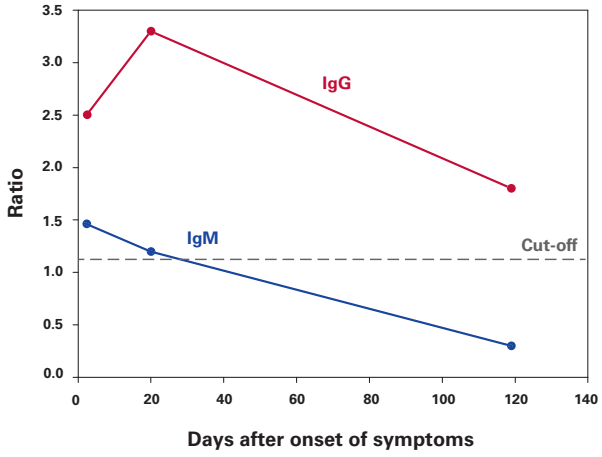
Days after onset of symptoms	EUROIMMUN Anti-Zika Virus ELISA	
	IgM (Ratio)	IgG (Ratio)
10	8.0	2.0
15	7.1	2.8
66	2.3	3.2
120	0.9	3.5
178	0.6	3.6



Patient returning from Brazil

- IgG and IgM levels positive after onset of symptoms. Slow titer decrease over several months.
- IgM level negative after approx. 3 weeks, IgG positive at this time point.

Days after onset of symptoms	EUROIMMUN Anti-Zika Virus ELISA	
	IgM (Ratio)	IgG (Ratio)
3	1.5	2.4
20	1.2	3.3
119	0.3	1.8

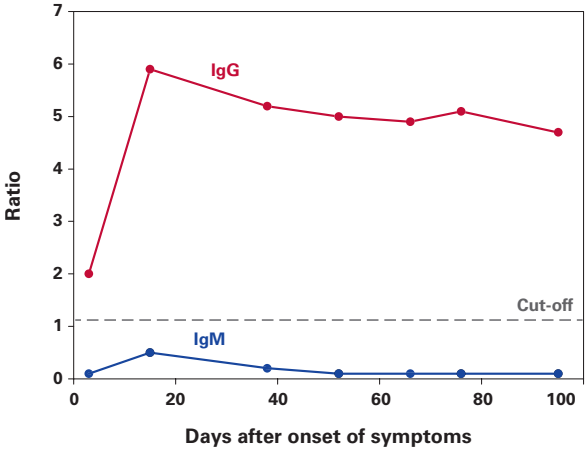


Case study: Patient from endemic region

Patient: 18 years old, male, Colombia

- IgM level negative over the whole period
- IgG level already positive at day 3 after onset of symptoms (literature: from day 5–6)

Days after onset of symptoms	EUROIMMUN Anti-Zika Virus ELISA	
	IgM (Ratio)	IgG (Ratio)
3	0.1	2.0
15	0.5	5.9
38	0.2	5.2
52	0.1	5.0
66	0.1	4.9
76	0.1	5.1
95	0.1	4.7



Semiquantitative test evaluation by ratio: Negative: Ratio < 0.8 Borderline: Ratio ≥ 0.8 to 1.1 Positive: Ratio ≥ 1.1

Indirect immunofluorescence

Alongside the ELISA test systems, EUROIMMUN offers indirect immunofluorescence tests (IIFT) for the diagnosis of Zika virus infections. There are three products available:

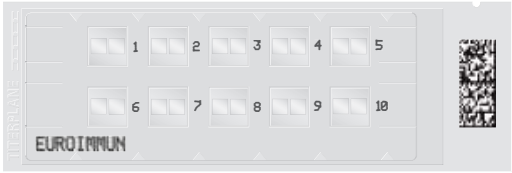
- **Anti-Zika Virus IIFT:** For the screening of Zika virus antibodies
- **Arbovirus Fever Mosaic 2:** For differential diagnosis of Zika, dengue, and chikungunya virus infections, which cause similar symptoms.
- **Arbovirus Profile 3:** Ideally suited for the investigation of cross reactivity within the group of flaviviruses.

Cells infected with different arboviruses are used as test substrate. In a positive reaction, mainly the cytoplasmic regions fluoresce, showing fine- to coarse-granular structures (see figure below). Some cells also show a net-like fluorescence pattern with a dense, perinuclear reactivity.

With these flavivirus mosaics/profiles, several specific antibodies can be detected simultaneously. They can be used to support clarification of cross reactivities between different flaviviruses and enable reliable differential diagnosis in the case of similar clinical symptoms.

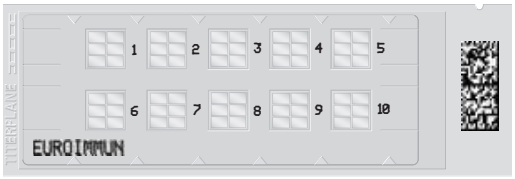
*ZIKV: Zika Virus, DENV: Dengue Virus, CHIKV: Chikungunya Virus, TBEV: tick-borne encephalitis virus, WNV: West Nile virus, JEV: Japanese encephalitis virus, YFV: Yellow fever virus

Anti-Zika Virus IIFT



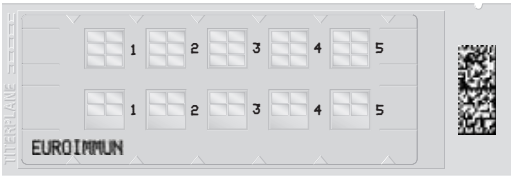
Substrates: Virus-infected cells: ZIKV* and non-infected cells

Arbovirus Fever Mosaic 2



Substrates: Virus-infected cells: ZIKV, CHIKV, DENV (Types 1-4)*

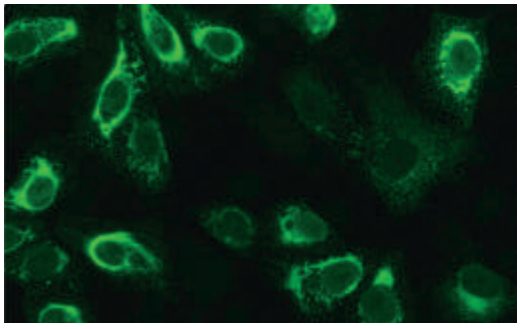
Arbovirus Profile 3



Substrates: Virus-infected cells: ZIKV, CHIKV, DENV (Type 1-4), TBEV, WNV, JEV, YFV*

Cross reactivity

Since complete virus particles are used as antigen in the IIFT products (contrary to the ELISA), cross reactivities are to be expected with the antibodies against viruses of the flavivirus family. In primary flavivirus infection, the dominant antibody titer of the virus which causes the infection can usually be identified by creating a dilution series of the patient sample. In secondary flavivirus infections, a high cross reactivity of the IgG antibodies must be expected. The final titers are equal or similar on all flavivirus substrates. In some cases, the determination of IgM antibodies may allow differentiation.



Fluorescence pattern: positive reaction: IgG antibodies against Zika virus.

Automation

ELISA

The Anti-Zika Virus ELISAs are suitable for processing on fully-automated analysis instruments. The tests are validated for the Analyzer I and Analyzer I-2P from EUROIMMUN and the DSX device from the manufacturer Dynex. Automated performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

IIFT

The IIF tests for the diagnosis of a Zika virus infection can be processed by the automated laboratory devices Sprinter and Sprinter XL from EUROIMMUN. All steps from the dilution and assignment of samples to the incubation and washing of slides are performed fully automatically. Evaluation of the incubated slides at the microscopes will soon be possible, supported by the computer-aided immunofluorescence microscope EUROPattern.

EUROIMMUN Analyzer I-2P

EUROIMMUN Sprinter XL

Product overview:

Test systems	Order number
Anti-Zika Virus ELISA (IgM)	EI 2668-9601 M
Anti-Zika Virus ELISA (IgG)	EI 2668-9601 G
Anti-Zika Virus IIFT (IgG or IgM)	FI 2668-#### G/M
Arbovirus Fever Mosaic 2 (IgG or IgM)	FI 2668-####-1 G/M
Arbovirus Fever Mosaic 2 EUROPattern (IgG or IgM)	FR 2668-####-1 G/M
Arbovirus Profile 3 (IgG or IgM)	FI 2668-####-3 G/M

Publications:

1. Huzly D, Hanselmann I, Schmidt-Chanasit J, Panning M. High specificity of a novel Zika virus ELISA in European patients after exposure to different flaviviruses. Euro Surveill. 2016;21(16):pii=30203
2. Granger D, Gomez LJ, Schimek M, Dubbels M, Mosquera JA, Christensen J, Bistodau S, Strain A, Theel ES. Zika virus Antibody Detection: Evaluation of Three Different Serologic Methodologies. Poster CVS 2016 (USA)
3. Frank C, Cadar D, Schlaphof A, Neddersen N, Günther S, Schmidt-Chansit J, Tappe D. Sexual transmission of Zika virus in Germany, April 2016. Euro Surveill. 2016; 21(23):pii=30252
4. Zanluca C, Dos Santos CN. Zika virus – an overview. Microbes Infect. 2016 May;18(5):295-301
5. Johansson MA, Mier-Y-Teran-Romero L, Reefhuis J, Gilboa SM, Hills SL. Zika and the Risk of Microcephaly. N Engl J Med. 2016 May 25
6. Musso D, Gubler DJ. Zika Virus. Clin Microbiol Rev. 2016 Jul;29(3):487-524
7. Zhang FC, Li XF, Deng YO, Tong YG, Qin CF. Excretion of infectious Zika virus in urine. Lancet Infect Dis. 2016 May 2
8. Driggers RW, Ho CY, Korhonen, et al. Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. N Engl J Med (2016) March
9. Fourcade C, Mansuya JM, Dutertre MD, Delobel B. Viral load kinetics of Zika virus in plasma, urine and saliva in a couple returning from Martinique, French West Indies. Journal of Clinical Virology, Jun 2016
10. Calleri G, Burdino E, Bonora S, Raso R. Zika virus infection in two travelers returning from an epidemic area to Italy, 2016: Algorithm for diagnosis and recommendations, Travel Medicine and Infectious Disease (2016)

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HI_2668_I_UK_B01, 07/2016