Serological differentiation of Zika from dengue virus infections

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SEROLOGICAL DIAGNOSIS OF ZIKA virus (ZIKV) infections is challenging due to the high cross reactivity between flaviviruses. Test systems utilising virus-specific antigen can overcome this obstacle. A novel anti-ZIKV ELISA based on a highly specific antigen is devoid of cross reactivity with dengue virus (DENV) antibodies, and provides highly specific and sensitive ZIKV diagnostics.

ZIKV infection

ZIKV is currently spreading rapidly in South and Central America and the Caribbean and is increasingly posing a risk in other parts of the world. The virus is transmitted primarily by mosquitos of the Aedes genus, which are ubiquitous in many tropical and non-tropical countries. It can also be spread by sexual contact. ZIKV infections manifest with fever, exanthema and arthritis, although in most cases the disease symptoms are mild. ZIKV infections can, however, cause severe neurological complications, in particular microcephaly in newborns and Guillain-Barré syndrome. ZIKV infections are difficult to differentiate clinically from infections with DENV and chikungunya virus (CHIKV), which are endemic in much the same geographical regions and manifest with similar symptoms. Therefore, laboratory testing plays an important role in differential diagnostics.

ZIKV serology

Serological tests supplement direct virus detection, and provide a much longer diagnostic window. Detection of the virus by RT-PCR in serum or plasma is only effective during the viraemic phase within the first week after onset of symptoms and may already be negative by the time a patient consults a doctor. Antibody detection is effective from soon after symptom onset (4 to 7 days) to beyond convalescence. Primary acute infections are generally characterised by the occurrence of IgM antibodies, with IgG appearing at the same time or shortly afterwards. IgM remain detectable for several months, while IgG are assumed to persist life-long. Detection of specific IgM antibodies or a rise in the specific IgG titer in a pair of samples taken at least 7 to 10 days apart is evidence of an acute infection. In a secondary flavivirus infection (e.g. previous vaccination or infection), IgM may be delayed, of reduced intensity or not detectable at all. Therefore, additional tests like the detection of IgG or neutralisation test are recommended.

In addition to their application in acute diagnostics, serological analyses are also useful for monitoring purposes, for example in prenatal diagnostics, sexual health care and epidemiological studies. Pregnant women with serological evidence of an infection can be offered intense prenatal monitoring, while seronegative women may be spared unnecessary worry. Due to the lengthy persistence of ZIKV in semen, men who have resided in or travelled to endemic regions are advised to abstain from unprotected sexual intercourse for six months after returning to prevent sexual transmission, especially when their partner is or could be pregnant. Serological testing can be helpful in these cases for excluding or identifying an infection. Furthermore, persons returning from endemic regions to ZIKV-free countries should not donate blood for a stipulated time period, for example in Germany defined as four weeks by the Paul-Ehrlich Institute. After this time, serological testing can verify the safety of donated blood products.

▼**TABLE 1:** 100% sensitivity in ZIKV RT-PCR-positive samples

n = 17		RT-PCR confirmed ZIKV infection
EUROIMMUN Anti-Zika Virus ELISA (IgM/IgG combined)	positive	17
	borderline	0
	negative	0

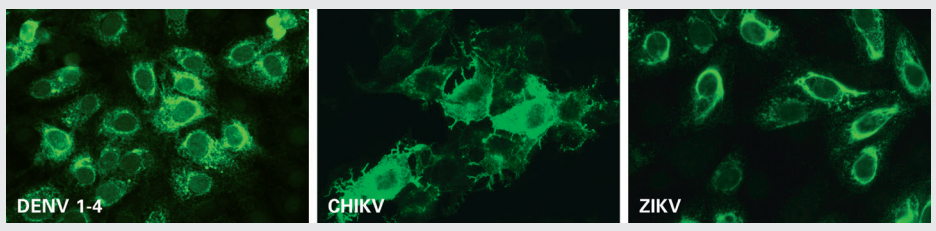
▼**TABLE 2:** 100% sensitivity in follow-up samples from ZIKV infected patients

n = 31		RT-PCR confirzmed ZIKV infection
EUROIMMUN Anti-Zika Virus ELISA (IgM/IgG combined)	positive	31
	borderline	0
	negative	0

▼**TABLE 3:** 96-100% specificity in patients with flaviviral and other infections/vaccinations

Cohort (in total >380 samples)	Anti-Zika Virus ELISA positive	
	IgM	IgG
DENV infection, high-level IgM	0%	0%
DENV infection, high-level IgG	0%	0%
DENV infection, further cases	0%	0%
TBEV infection/vaccination	0%	0%
WNV infection	2.9%	0%
JEV infection	0%	4.0%
Yellow fever vaccination	0%	0%
CHIKV infection	0%	0%
Plasmodium infection (past)	1.4%	0%

▼**FIGURE 1.** Positive reactions in IIFT for DENV, CHIKV and ZIKV



Highly specific ZIKV ELISA

A highly specific anti-ZIKV ELISA has been developed based on recombinant non-structural viral protein 1 (NS1) from ZIKV. Use of this virus-specific antigen avoids the cross reactivity typically associated with tests based on whole virus antigens or viral glycoproteins. A recently published multicohort study has confirmed the exceptional specificity of the ELISA, in particular with respect to the important differential diagnostic parameters DENV and CHIKV.

In the study, anti-ZIKV antibodies of classes IgM and IgG were analysed in sera from patients with RT-PCR confirmed or suspected ZIKV infections, patients with DENV, CHIKV, West Nile virus (WNV), Japanese encephalitis virus (JEV) or Plasmodium infections, yellow-fever vaccinated individuals and healthy blood donors. In 17 RT-PCR confirmed ZIKV specimens from patients with active or late ZIKV infection (samples collected at least six days after symptom onset, Table 1), the ELISA sensitivity amounted to 100% for a combination of IgM and IgG. In the suspected ZIKV cases, the combined sensitivity amounted to 90%. In an additional investigation using samples from 31 patients with RT-PCR confirmed ZIKV infections (follow-up samples taken 7-10 days after positive RT-PCR result, Table 2), the ELISA demonstrated a sensitivity of 100% for IgM/IgG.

The specificity of the ELISA with respect to the blood donors was 99.8%. Cross reactivity with high-level anti-dengue virus antibodies was not detectable (Table 3). Among the patients with potentially cross-reactive antibodies, overall anti-Zika positive rates were 0.8% for IgM and 0.4% for IgG. In a further published study the absence of cross reactivity was confirmed in patients with DENV infections or other viral infections or vaccinations.

Differential diagnostics by IIFT

An alternative serological ZIKV test based on indirect immunofluorescence is also suitable for differential diagnosis of ZIKV, DENV and CHIKV infections. The IIFT Arbovirus Fever Mosaic 2 utilises cells infected with ZIKV, DENV (serotypes 1, 2, 3 and 4) or CHIKV as the antigenic substrates. The BIOCHIP substrates are incubated in parallel, and results are evaluated by fluorescence microscopy (Figure 1). However, due to the use of whole virus particles, cross reactivities between flavivirus antibodies (e.g. DENV and ZIKV) must be taken into account.

Perspectives

Although no longer classified as a public health emergency, ZIKV is viewed by the World Health Organisation (WHO) as an enduring public health challenge requiring a global management strategy. It is anticipated that ZIKV will continue to spread to areas where there are mosquitos able to carry it. The highly specific Anti-Zika Virus ELISA described here enables reliable serological diagnostic differentiation of ZIKV infections from the geographically overlapping and clinically similar DENV and CHIKV infections. With its high-throughput format and automatability, it can be employed in routine laboratories in endemic settings. The ELISA is, moreover, useful for surveillance and epidemiological studies, which are critical for advancing understanding of the risks and complications associated with the disease.