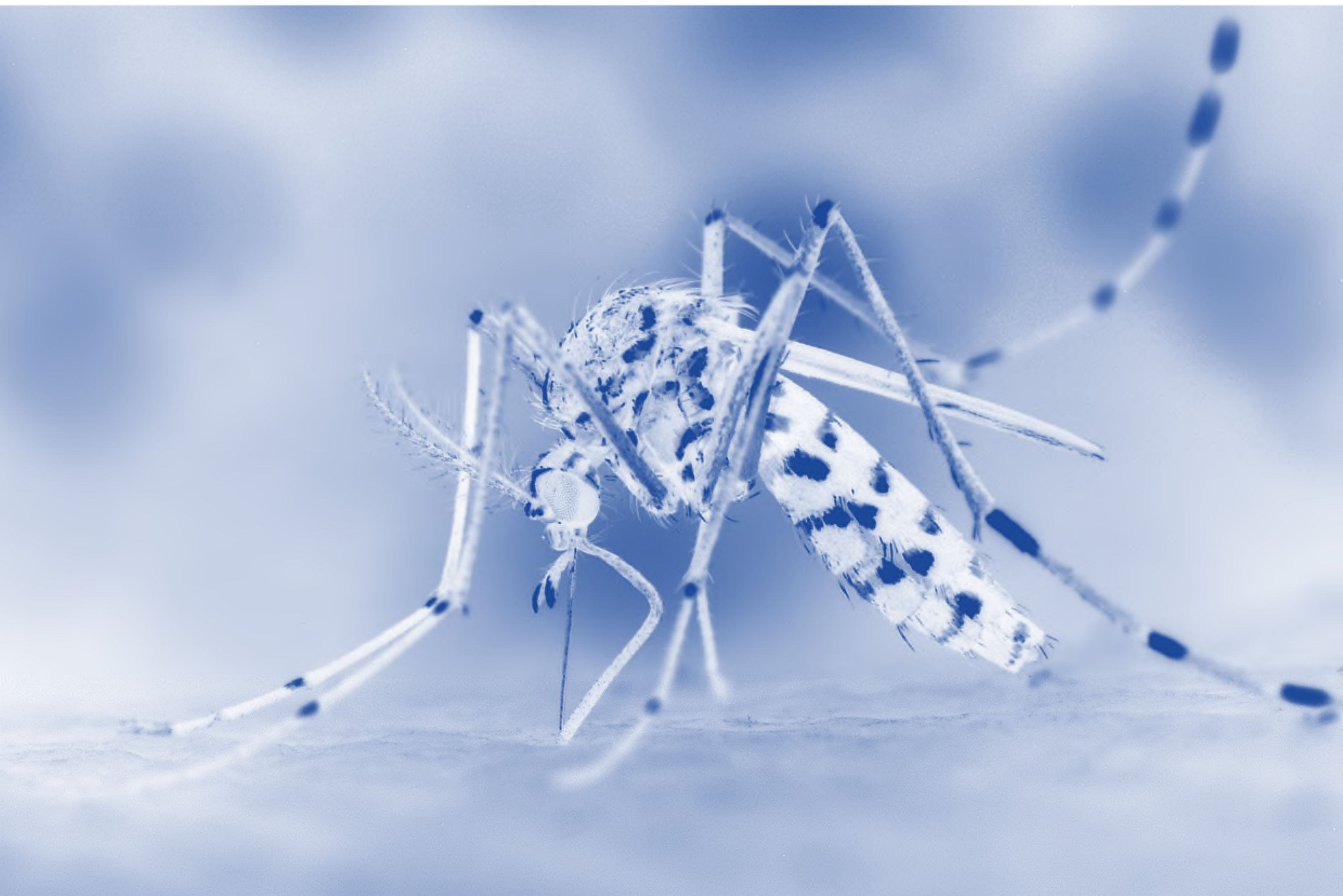
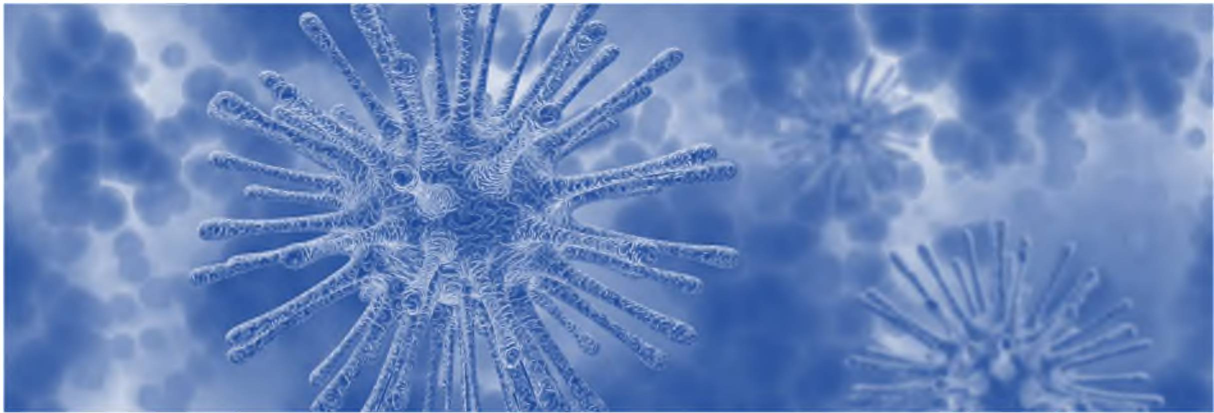
ROBOSCREEN
DIAGNOSTICS

Dengue Virus specific IgM/ IgG ELISAs Reliable detection of acute and past dengue virus infections



Dengue Virus specific IgM/ IgG ELISAs Reliable detection of acute and past dengue virus infections



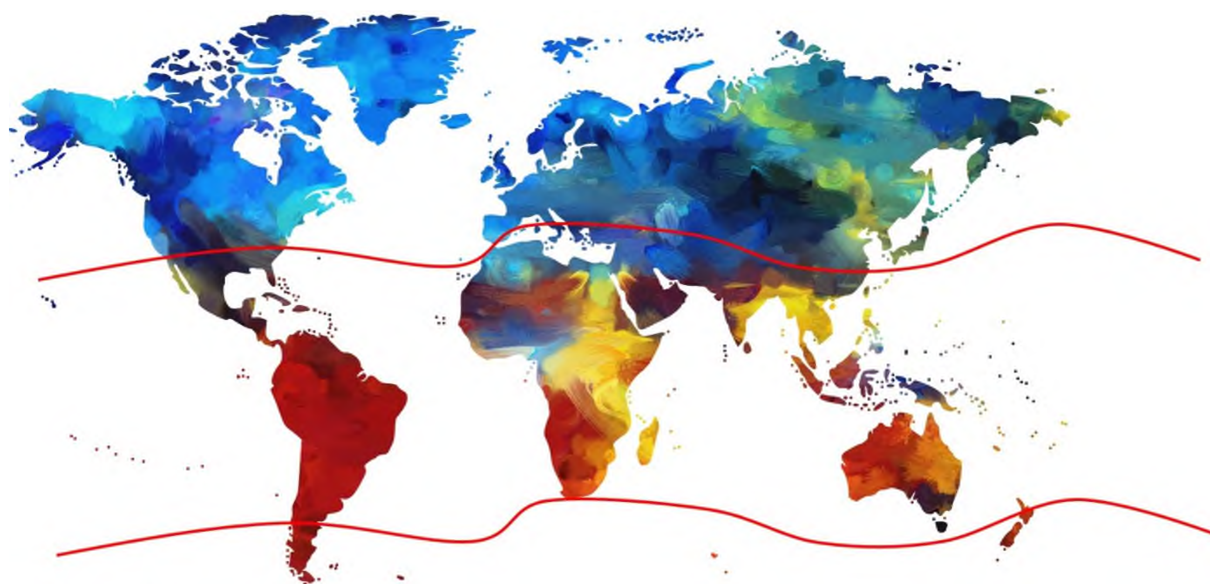
The dramatic increase in the incidence, mortality and geographical spread of dengue fever in recent decades has made this disease one of the greatest global health challenges. Rapid and accurate diagnosis of dengue is critical for clinical care, especially for early detection of severe cases, case confirmation and differential diagnosis with other infectious diseases. Moreover, it is also important for surveillance activities, outbreak monitoring, pathogenesis studies, academic research, vaccine development, and clinical trials. The Dengue virus specific IgM/ IgG ELISAs offered by Roboscreen GmbH provide a sensitive and highly specific solution for reliable detection of anti-DENV IgM/ IgG antibodies in human serum and plasma samples.

Dengue virus specific IgM/ IgG ELISA by Roboscreen GmbH

- patented (WO2015/139784) serological assay, developed in cooperation with the Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany
- strongly reduced cross-reactivity to heterologous flaviviruses like ZIKV, WNV, YFV, CHIKV and TBEV
- CE-IvD labeled

Dengue Fever

Dengue fever is a mosquito-borne viral disease caused by an infection with the dengue virus (DENV). It is also known as breakbone fever due to the severity of muscle spasms and joint pain. The global incidence of dengue has grown dramatically in recent decades. The infection is now endemic in tropical and subtropical regions of the world. Each year 400 million dengue infections are estimated to occur worldwide, with about 96 million resulting in illness [1]. Although the disease is usually mild and self-limiting, a few people develop severe forms including hemorrhagic fever and shock, that can be life threatening.



Dengue virus belongs to the Flaviviridae family and is transmitted by the bite of female mosquitoes mainly of the species Aedes aegypti and, to a lesser extent, Aedes albopictus. [2] These mosquitoes are also vectors of Chikungunya, Yellow Fever, West Nile and Zika virus. Dengue causes a wide spectrum of disease. This can range from subclinical, symptomless disease to severe flu-like symptoms in those infected. Symptoms of dengue fever include severe joint and muscle pain, swollen lymph nodes, headache, fever, exhaustion, and rash. The presence of fever, rash, and headache (the „dengue triad") is characteristic for dengue fever. [3] There are four distinct but closely related serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4). Recovery from infection provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial, and temporary. Subsequent infections (secondary infection) caused by other serotypes increase the risk of developing severe dengue. Severe dengue is a rare complication called dengue hemorrhagic fever, characterized by high fever, damage to lymph and blood vessels, bleeding from the nose and gums, petechiae (small red spots or purple splotches or blisters under the skin), enlargement of the liver, and failure of the circulatory system. This form of dengue fever can be life threatening and can progress to the most severe form of the illness, dengue shock syndrome (DSS). [1-3] The prognosis for dengue is usually good. The worst symptoms of the illness typically last 1 to 2 weeks, and most patients will fully recover within several additional weeks. Typical dengue infection is fatal in less than 1 % of cases; however, the more severe dengue

hemorrhagic fever is fatal in 2.5 % of cases. If dengue hemorrhagic fever is not treated, mortality (death) rates can be as high as 20-50 %: [3] Diagnosis of dengue infection is usually made when a patient exhibits the typical clinical symptoms and has a history of being in an area where dengue is endemic. Nevertheless, dengue fever can be difficult to diagnose because its symptoms overlap with those of many other viral illnesses and tropical diseases, such as West Nile and chikungunya fever. A secured diagnosis of a DENV infection can be made by use of virological tests, that directly detect elements of the virus (RT-PCR; Dengue Virus NS1-Antigen test), and serological tests, which detect human-derived antibodies that are produced in response to the virus: [1-3] Depending on the time of patient presentation, the use of different diagnostic methods is advisable. Patient samples collected during the first week of illness should be tested by both serological and virological methods (RT-PCR). Serological methods, such as enzyme-linked immunosorbent assays (ELISA), may confirm the presence of a recent or past infection, with the detection of IgM or IgG anti-dengue antibodies. IgM antibodies are detectable approximately 1 week after infection and are highest at 2 to 4 weeks after the onset of illness. They remain detectable for about 3 months. The presence of IgM is indicative of a recent DENV infection. IgG antibody levels take longer to develop than IgM, but IgG remain in the body for years. The presence of IgG is indicative of a past infection. [3] There is no specific treatment for dengue fever. Fever reducers and pain killers can be taken to control the symptoms of muscle aches, pains and fever. For severe dengue, medical care by experienced physicians and nurses can save lives – decreasing mortality rates from more than 20 % to less than 1 %. [1]

References

1. WHO, Factsheet Dengue and severe dengue, 4th November 2019. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
2. ECDC, Factsheet about dengue, accessed 10th December 2019. https://www.ecdc.europa.eu/en/dengue-fever/facts/factsheet
3. John P. Cunha, “Dengue Fever”, MedicineNet, accessed 10th December2019.https://www.medicinenet.com/dengue_fever/article.htm

A unique assay structure

Strongly reduced cross-reactivity to heterologous flaviviruses

The pitfalls of diagnosing a dengue infection

Arthropod transmitted flaviviruses are endemic to many parts of the world and include a large number of important human pathogens, such as Dengue, Zika, Yellow Fever, Chikungunya, West Nile, and Tick-borne encephalitis virus (DENV, ZIKV, YFV, CHIKV, WNV, and TBEV), respectively. The specific diagnosis of DENV infections via antibody detection is severely complicated due to the high degree of cross-reactivity displayed by antibodies against heterologous flaviviruses. Co-circulation of several flaviviruses and vaccinations against some of them e.g: YFV or TBEV, contribute to the considerably more difficult diagnosis and surveillance of these infections. Especially, the massive co-circulation of ZIKV in areas endemic for DENV, makes a specific diagnosis and differentiation of these infections mandatory. Antibodies against ZIKV are particularly cross-reactive to DENV and vice versa due to conserved parts of their proteins. Most flavivirus cross-reactive antibodies are produced against the highly conserved fusion loop (FL) domain in the viral envelope (E) protein. The E-Protein is a major target of the human antibody response during DENV infections and is used in most available tests. Consequently, currently available tests are severely compromised by cross-reacting antibodies and cannot rule out false positive results. Therefore, positive test results currently have to be confirmed by virus neutralization tests, which are time consuming and require BSL-3 laboratories. [4-5]

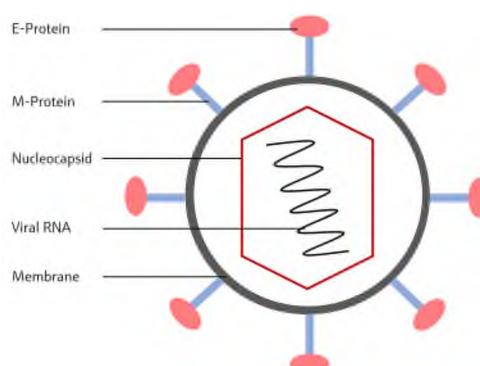
To overcome these time-consuming test procedures, the Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany, developed a patented unique assay concept (WO2015/139784) for the sensitive and specific serological diagnosis of DENV-infections. In a partnership with Roboscreen GmbH, a manufacturer of IvD-diagnostics in Leipzig, Germany, the test system was brought to market and complies with the standards for IvD Diagnostics. For the special antigen coating of the DENV specific IgM/IgG ELISAs, insect cell derived recombinant E-proteins of the four DENV-serotypes (DENV 1-4) with amino acid point mutations in the fusion loop and an adjacent loop domain of the E-Protein were generated. By use of specific mixtures of these mutant antigens and a pre-incubation step with a specific blocking reagent for the DENV IgG ELISA, the binding of cross-reactive antibodies from heterologous flaviviruses like ZIKV, YFV, WNV, CHIKV and TBEV is strongly reduced. This guarantees a sensitive and specific diagnosis of DENV infected serum and plasma samples using IgG or IgM measurements. [4-5]

References

4. Rockstroh, A. et al., 2015. Recombinant Envelope-Proteins with Mutations in the Conserved Fusion Loop Allow Specific Serological Diagnosis of Dengue-Infections. PLoS Negl Trop Dis. Nov 13, 2015, p. 9(11):e0004218. doi: 10.1371/journal.pntd.0004218. eCollection 2015 Nov.
5. Rockstroh, A. et al., 2017. Specific detection of dengue and Zika virus antibodies using envelope proteins with mutations in the conserved fusion loop. Emerg Microbes Infect. Nov 8, 2017, p. 6(11):e99. doi: 10.1038/emi.2017.87.
6. Rodriguez , AK et al., 2019. Molecular characteristics and replication mechanism of dengue, zika and chikungunya arboviruses, and their treatments with natural extracts from plants: An updated review. EXCLI J. 2019 Oct 31;18:988-1006. doi: 10.17179/excli2019-1825. eCollection 2019.

Structure of the dengue virus

The Dengue virus is a spherical enveloped virus with approximately 40-60 nm in diameter. It has a single stranded positive-sense RNA genome, which is surrounded by an icosahedral nucleocapsid and covered by a lipid membrane that contains the viral E- and M-glycoproteins. Incorporation of the virions to the host cells is mediated by the interaction of the viral envelope glycoprotein (E) with receptors on the surface of the target cell. Via endocytosis the virions are internalized. By fusion of the viral envelope and endosomal membrane, the nucleocapsid is released into the cytosol and the viral replication is started. After virus assembly in the endoplasmatic reticulum (ER) and Golgi-System, mature viruses are released from the host cell by exocytosis and can subsequently infect more cells. [6]



A unique assay structure

Strongly reduced cross-reactivity to heterologous flaviviruses

Mutated DENV E-Proteins allow specific detection of dengue in an IgG ELISA

To enhance specificity of serological DENV diagnosis, 4 amino acid point mutations were inserted into the conserved fusion loop (FL) domain and an adjacent loop domain of DENV wildtype E proteins (DENV Ewt), yielding quadruple mutants for DENV serotypes 1 to 4 (DENV 1–4 Equad proteins). [4] Sera from 38 DENV-, 13 WNV-, 19 TBEV- infected, 8 YFV vaccinated and 7 uninfected individuals were incubated with DENV-2 Ewt, Fig. 1A), DENV-2 Equad protein (Fig. 1B) or DENV 1-4 Equad Mix (Fig. 1C) to compare sensitivity and specificity of the different antigens. Strong binding for DENV-positive samples was observed for DENV-2 Ewt (Fig 1A), however, several WNV and TBEV- positive sera showed cross-reactive signals which were in the range of DENV-infected sera. When using the DENV-2 Equad protein, the cross-reactivities of WNV- and TBEV infected samples were significantly reduced (Fig. 1B). The use of a mixture of the Equad proteins of all four serotypes (DENV 1–4 Equad Mix) resulted in an increase of the 25th percentile from 1.36 to 1.6, demonstrating a higher sensitivity compared to using only DENV-2 Equad. At the same time, cross-reactivity of WNV- and TBEV-positive sera was even further reduced showing a higher specificity of the test in comparison to using DENV-2 Equad only. [4]

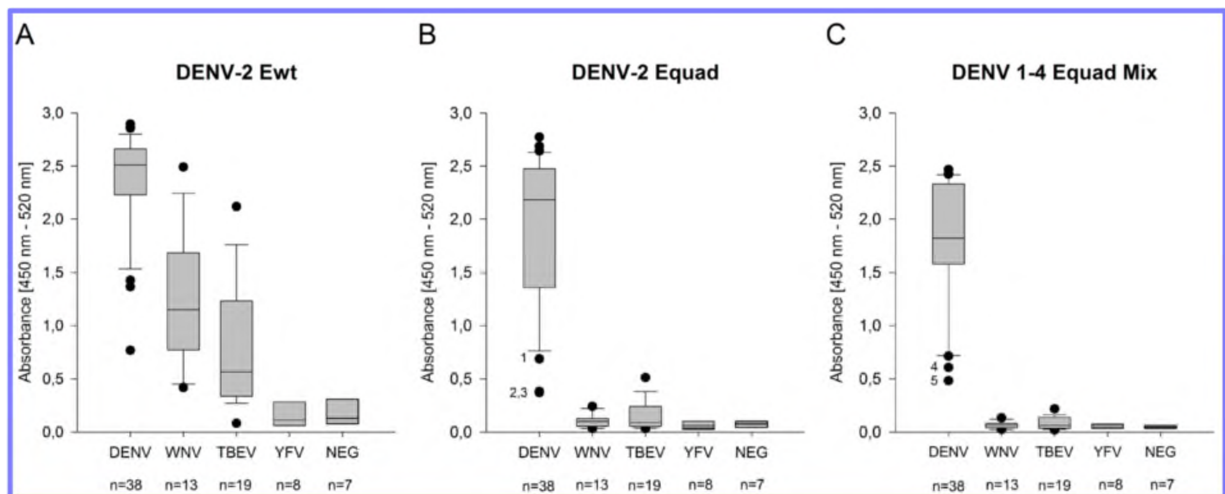


Figure 1: DENV-2 Ewt (A), DENV-2 Equad (B) and a DENV 1–4 Equad mixture (C) were tested with DENV-, WNV- and TBEV-infected and YFV-vaccinated sera compared to negative (NEG) samples in an IgG-ELISA (n = number of individuals). Bottom and top of the boxes are the first and third quartiles. The median signal is depicted as a line inside the box. Whiskers represent the 9th and the 91st percentile. Outliers in B and C are marked with numbers (1–5). Measurements were performed in duplicates in at least two independent experiments.

A unique assay structure

Strongly reduced cross-reactivity to heterologous flaviviruses

Mutated DENV E-Proteins strongly reduce cross-reactivity against WNV and TBEV in an IgG ELISA

To compare these results with a state-of-the-art diagnostic method, a number of the serum samples were analyzed with a commercially available DENV-IgG ELISA (Panbio Indirect IgG ELISA), the DENV Ewt protein and the DENV 1-4 Equad mix (Fig. 2). All 3 antigens detected 3/3 DENV-positive sera as positive and 3/3 negative sera as negative. However, substantial cross-reactivity for WNV and TBEV was observed for the commercially available DENV-IgG ELISA and the DENV Ewt protein. In contrast, when using the DENV 1–4 Equad mixture high signals were obtained only with the three DENV-infected sera and cross-reactivity was strongly reduced in all samples from WNV- and TBEV-infections (Fig. 2). [4]

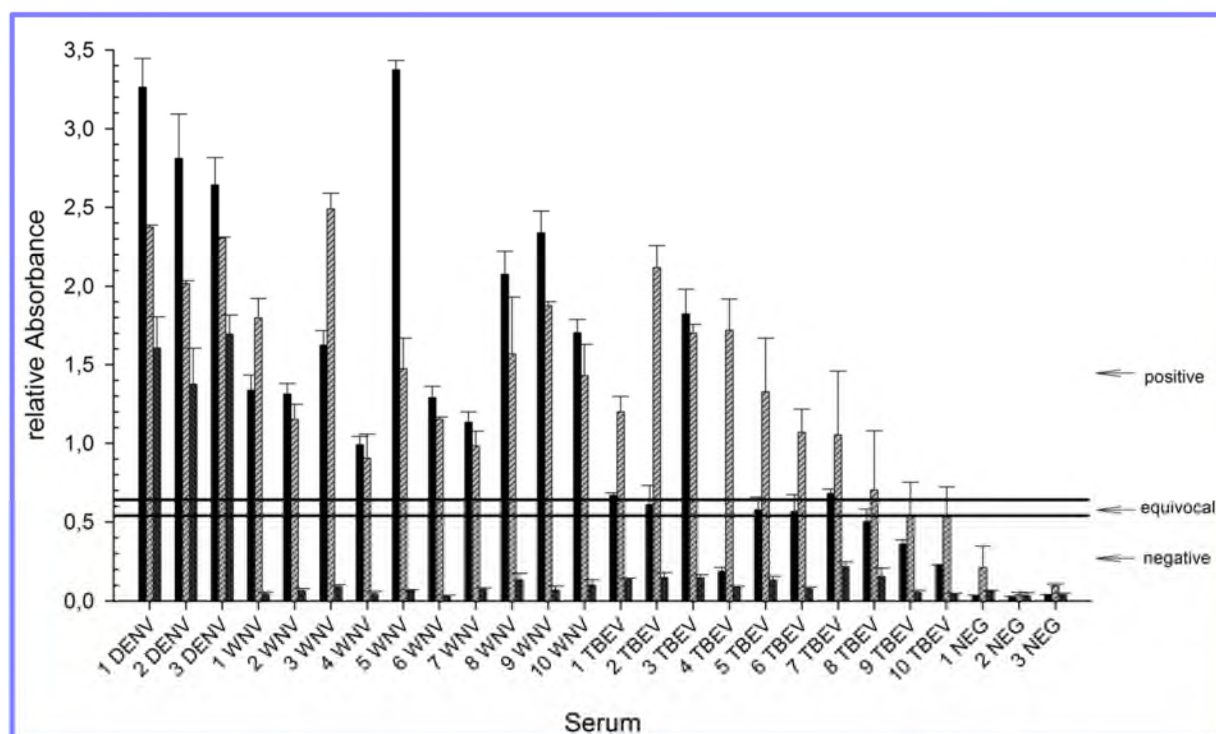


Figure 2: Comparison of different antigens for the detection of DENV IgG. Sera positive for IgG against DENV, WNV, TBEV or negative control sera were analyzed with the Panbio Indirect IgG ELISA (black), the DENV-2 Ewt protein (light gray, lined) or the DENV1-4 Equad mix (dark grey, lined). The absolute absorbance is indicated. Cut-Off values for the Panbio test were obtained by calculation of the internal standard of the manufacturer; these are indicated at the right and only refer to this test (horizontal bars: DENV-positive results with an OD-value higher than 1.1*cut-off, equivocal results having an OD-value between 1.1*cut-off and 0.9*cut-off, negative results with an OD-value lower than 0.9*cut-off).

References
4. Rockstroh, A. et al., 2015. Recombinant Envelope-Proteins with Mutations in the Conserved Fusion Loop Allow Specific Serological Diagnosis of Dengue-Infections. PLoS Negl Trop Dis. Nov 13, 2015, p. 9(11):e0004218. doi: 10.1371/journal.pntd.0004218. eCollection 2015 Nov.

A unique assay structure

Strongly reduced cross-reactivity to heterologous flaviviruses

Mutated DENV E-Proteins strongly reduce cross-reactivity against WNV and TBEV in an IgM ELISA

The antigens DENV-2 Ewt and DENV 1-4 Equad mix were subsequently used to measure IgM antibodies in IgM positive human DENV- and WNV-sera (Fig. 3). Generally, the cross-reactivity of heterologous flavivirus IgM antibodies was lower than for IgG, as demonstrated by less binding to the Ewt protein (Fig. 3A). By using the DENV 1–4 Equad mixture the mean value of signals for DENV sera was enhanced as compared for DENV-2 Ewt. On the other hand, WNV crossreactivity was significantly reduced in comparison to DENV-2 Ewt. [4]

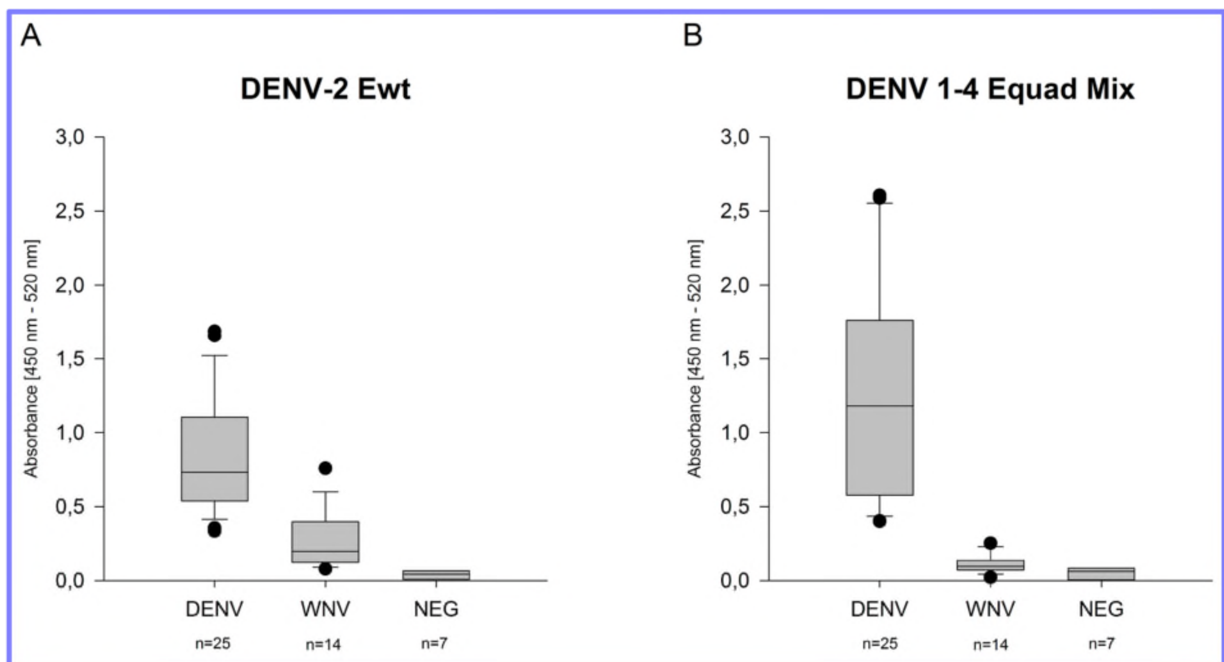
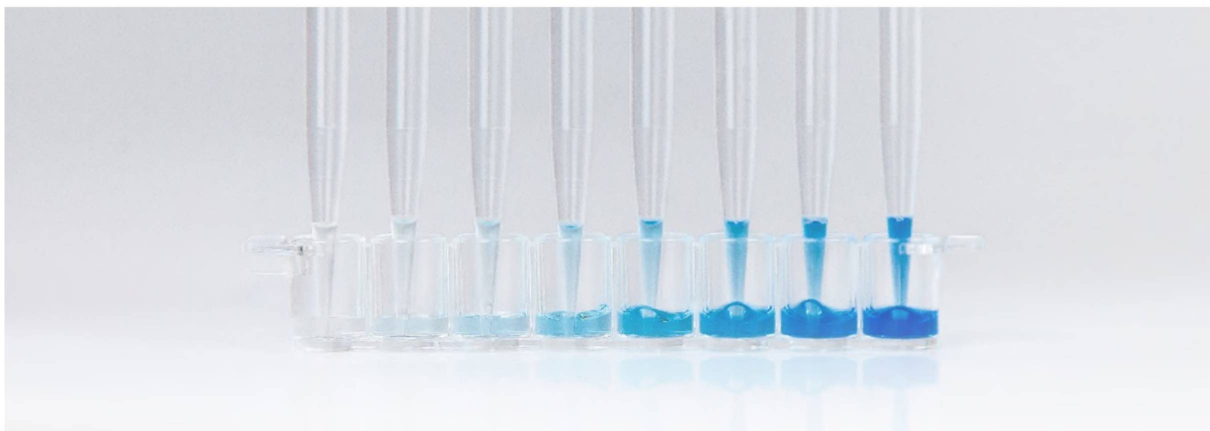


Figure 3: IgM-ELISA of DENV-2 Ewt (A) and DENV 1–4 Equad mixture (B) with different DENV, WNV and negative (NEG) sera (n = number of individuals). Bottom and top of the boxes are the first and third quartiles. The median signal is depicted as a line inside the box. Whiskers represent the 9th and the 91st percentile. Measurements were performed in duplicates in at least two independent experiments.



A unique assay structure

Strongly reduced cross-reactivity to heterologous flaviviruses

An IgG competition ELISA using DENV mutated E-Proteins specifically distinguishs DENV and ZIKV

Cross-reactivity is a long-known complication in the serological diagnosis of flavivirus-infections. The ZIKA virus poses a particular problem in the diagnosis of Dengue. On the one hand due to the massive co-circulation of ZIKV and DENV, on the other hand because antibodies to ZIKV are particularly cross-reactive towards DENV due to the conserved parts of their proteins and vice versa.[5] To facilitate a specific serological differentiation between DENV and ZIKV infections, four amino acid point mutations were inserted in the conserved fusion loop (FL) domain of the ZIKV E-protein (Equad) and compared to the DENV 1–4 Equad mixture, which was shown to significantly reduce cross-reactivities in dengue serological diagnosis [4]. A competition ELISA setup was chosen to further reduce the IgG cross-reactivity between DENV- and ZIKV- positive sera. Therefore, DENV- and ZIKV- IgG-positive as well as the negative sera were measured on DENV 1–4 Equad after competition with ZIKV Equad (Fig. 4A) and on ZIKV Equad

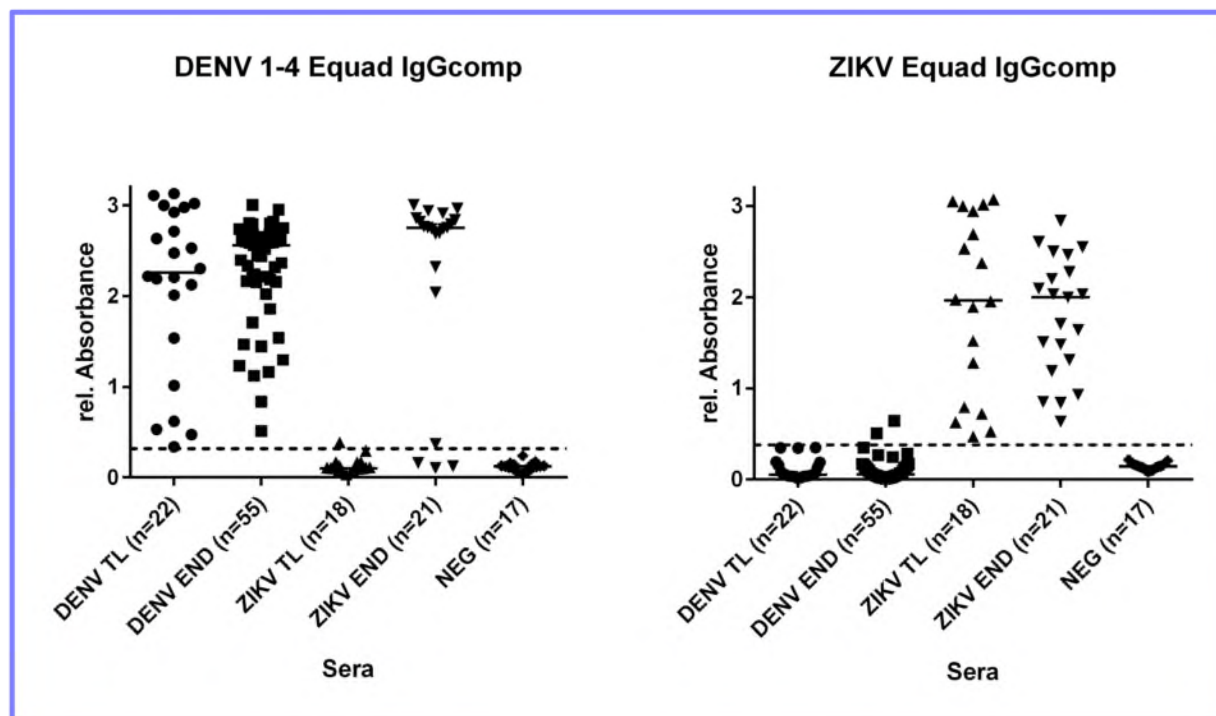


Figure 4: IgG Competition ELISA: Sera were measured on (A) DENV 1–4 Equad with ZIKV Equad competition and (B) ZIKV Equad with DENV 1–4 competition. One sample per patient was examined in two independent experiments and plotted as a mean data point. The dashed lines represent cutoffs determined by a ROC analysis with negative sera as controls.

References
4. Rockstroh, A. et al., 2015. Recombinant Envelope-Proteins with Mutations in the Conserved Fusion Loop Allow Specific Serological Diagnosis of Dengue-Infections. PLoS Negl Trop Dis. Nov 13, 2015, p. 9(11):e0004218. doi: 10.1371/journal.pntd.0004218. eCollection 2015 Nov.5. Rockstroh, A. et al., 2017. Specific detection of dengue and Zika virus antibodies using envelope proteins with mutations in the conserved fusion loop. Emerg Microbes Infect. Nov 8, 2017, p. 6(11):e99. doi: 10.1038/emi.2017.87.

Dengue virus specific IgM/ IgG ELISAs

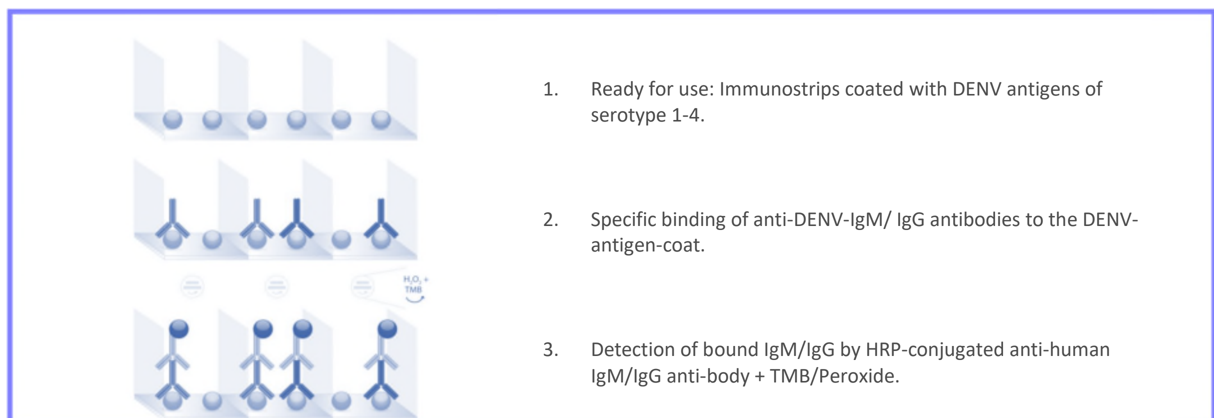
Strongly reduced cross-reactivity to heterologous flaviviruses

Strongly reduced cross-reactivity to other arboviruses

- **CE-IVD marked ELISAs** for detection of human IgM/IgG-antibodies against a Dengue virus infection in human serum and plasma samples
- **Strongly reduced cross-reactivity** against heterologous Arboviruses (ZIKA Virus, Yellow Fever Virus, West Nile Virus, Chikungunya-Virus, Tickborne encephalitis virus)
- **Flexible use** depending on sample throughput: standardized ELISA format with 12 x 8 reactions
- **Patented unique assay** concept (WO2015/139784) for the sensitive and specific serological diagnosis of DENV-infections
- **Specific antigen-coating** of ELISA strips with mutated recombinant E-proteins of the four DENV serotypes (DENV 1-4) strongly reduced cross-reactivity to heterologous flaviviruses like ZIKV, YFV, WNV, CHIKV and TBEV

Product specifications

	DENV IgM ELISA	DENV IgG ELISA
Target	Human anti-DENV IgM antibodies	Human anti-DENV IgG antibodies
Detection principle	Indirect ELISA	Indirect ELISA
Starting material	Human plasma and serum	Human plasma and serum
Detection time	3 h	4 h
Precision (CV %)	< 10 %	< 15 %
Linearity	1:10.000 sample dilution	1:10.000 sample dilution
Diagnostic sensitivity	94.1 %	98.4 %
Diagnostic specificity	99.3 %	98.6 %



DENV IgM ELISA

Analytical validation

Reproducibility of Dengue virus specific IgM ELISA was carried out on 8 DENV positive samples by testing 10-fold dilutions of the samples on 3 successive days and measurement of optical density at 450-620 nm. The ELISA showed a very small variation < 10 % for undiluted samples and < 20 % for a sample dilution up to 1:100.

Clinical validation

233 clinical samples were included in the validation, of which 86 were samples from demonstrably DENV infected patients and 147 samples from patients without DENV infection. The Dengue virus specific IgM ELISA showed a sensitivity of 94.1 % with a specificity of 99.3 %. The area under the ROC curve (AUC) was 97.9 % with $p<0.001$ (Figure 5).

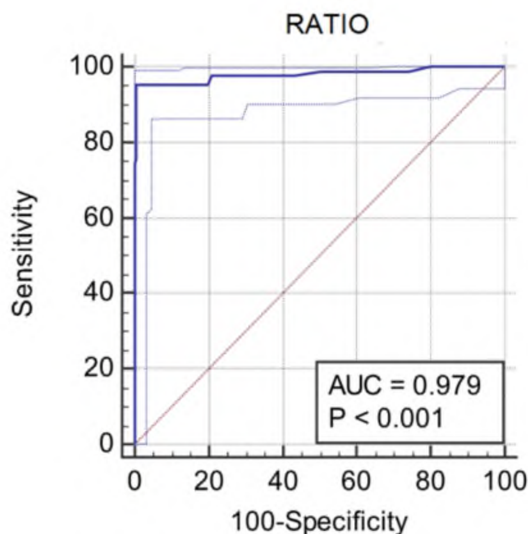


Figure 5: ROC-Analysis for DENV IgM ELISA. AUC: Area under the curve.

DENV IgG ELISA

Analytical validation

Reproducibility of Dengue virus specific IgG ELISA was carried out on one DENV positive sample with 3 semi-logarithmic dilution stages and on two patient samples with a pre-dilution of 1:100 in Assay buffer with 6-fold repeat on 6 different immunostrips D1. The measurement at the optical density at 450-620 nm was evaluated. The ELISA showed a very small variation < 10 % over all measurements.

Clinical validation

130 clinical samples were included in the validation, of which 61 were samples from demonstrably DENV infected patients and 69 samples from patients without DENV infection. The Dengue virus specific IgG ELISA showed a sensitivity of 98.4 % with a specificity of 98.6 %. The area under the ROC curve (AUC) was 98.9 % with $p < 0.001$ (Figure 6).

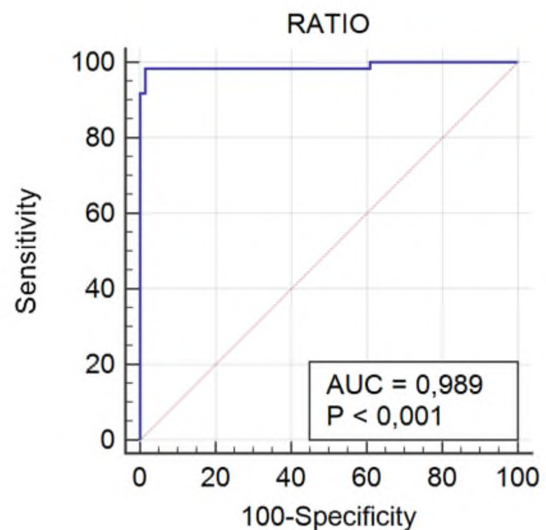


Figure 6: ROC-Analysis for DENV IgG ELISA. AUC: Area under the curve.

Order Information

Order number	Description	Quantity
847-0108000105	DENV IgM ELISA	12x8 reactions
847-0108000106	DENV IgG ELISA	12x8 reactions

Contact

Roboscreen GmbH
Hohmannstrasse 7
04129 Leipzig
Germany

Phone +49 341 989 734 0
FAX +49 341 989 734 199

info@roboscreen.com
www.roboscreen.com