# 3 Reliable Serological Testing for the Diagnosis of Emerging Infectious Diseases

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**Abstract**

Climate change, increased urbanization and international travel have facilitated the spread of mosquito vectors and the viral species they carry. Zika virus (ZIKV) is currently spreading in the Americas, while dengue virus (DENV) and chikungunya virus (CHIKV) have already become firmly established in most tropical and also many non-tropical regions. ZIKV, DENV and CHIKV overlap in their endemic areas and cause similar clinical symptoms, especially in the initial stages of infection. Infections with each of these viruses can lead to severe complications, and co-infections have been reported. Therefore, laboratory analyses play an important role in differential diagnostics. A timely and accurate diagnosis is crucial for patient management, prevention of unnecessary therapies, rapid adoption of vector control measures, and collection of epidemiological data.

There are two pillars to diagnosis: direct pathogen detection and the determination of specific antibodies. Serological tests provide a longer diagnostic window than direct methods, and are suitable for diagnosing acute and past infections, for disease surveillance and for vaccination monitoring. ELISA and indirect immunofluorescence test (IIFT) systems based on optimized antigens enable sensitive and specific detection of antibodies against ZIKV, DENV and CHIKV in patient serum or plasma. In recent years, Euroimmun (Lübeck, Germany) has developed numerous test systems for the serological diagnosis of (re-) emerging diseases, including a very sensitive and specific anti-ZIKV ELISA.

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**Keywords**

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## 3.1 Laboratory Testing for ZIKV Infections

Diagnostic testing for ZIKV infection is accomplished using mainly genome detection and serological methods [48, 63, 71, 91]. Recently, a number of in-house and commercial *in vitro* diagnostic assays for direct and indirect ZIKV detection have been developed and widely applied in routine laboratories [95, 96]. However, no single test is capable of accurately diagnosing ZIKV infections over the whole course of disease. Rather, a combination of direct and indirect detection methods is preferable to either approach alone, with serological methods being effective from soon after clinical onset to beyond convalescence. In the differential diagnosis of ZIKV infections, evaluation for DENV and CHIKV should be included because of their clinical similarity and co-endemicity [60]. Careful result interpretation is crucial as it will guide the clinical management regarding, for example, possible adverse pregnancy outcomes.

### 3.1.1 Direct ZIKV Detection

Only within the first week after symptom onset, ZIKV can be isolated from infected individuals via cell culture [2, 28, 33, 56]. Within the same time frame, highly specific reverse transcription-polymerase chain reaction (RT-PCR) assays allow for the detection of ZIKV RNA in serum samples [15, 46] (Fig. 3.1a, b). In saliva, urine, semen or amniotic fluid, the viral genome may be detectable for a prolonged time [4, 16, 37, 72]. In contrast to DENV diagnostics, where the determination of virus antigen is a common approach, ZIKV antigen assays are not yet available [54]. Due to the short viremic phase, the high number of asymptomatic infections and the frequent difficulty in precisely determining the onset date of

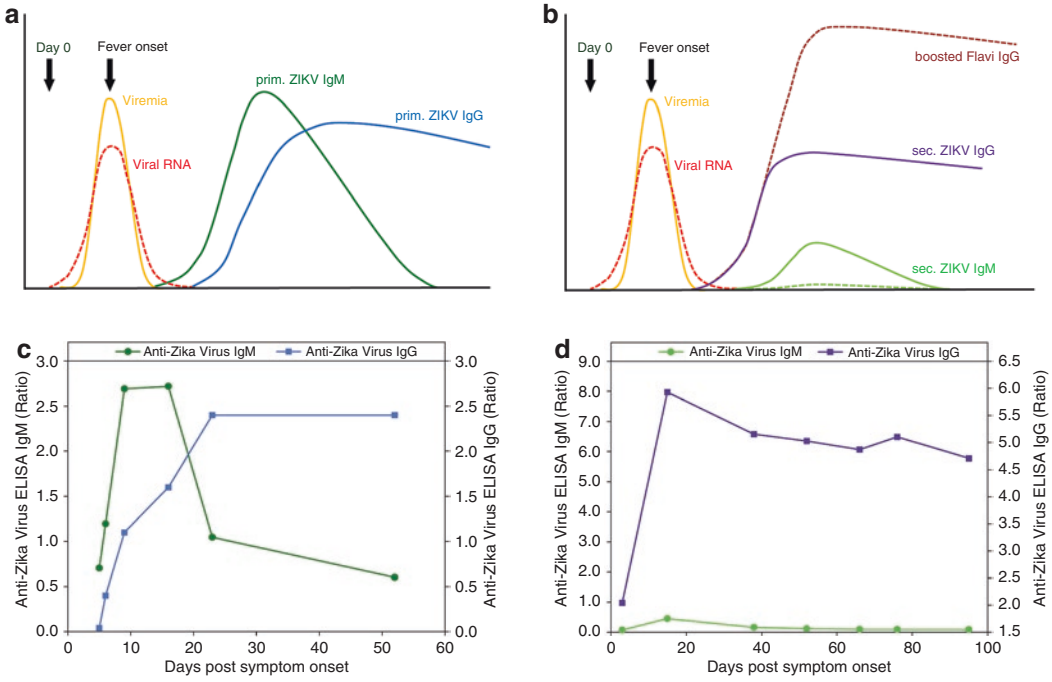


**Fig. 3.1** Appearance of the major laboratory diagnostic markers in (**a**) primary ZIKV infections and (**b**) secondary flavivirus infections with ZIKV: virus isolation, RNA detection, and anti-ZIKV antibodies (IgM, IgG). Titer course of anti-ZIKV IgM and IgG in (**c**) a European traveler after primary infection with ZIKV, and (**d**) a Columbian resident who contracted ZIKV as secondary flavivirus infection

symptoms, the utility of direct ZIKV detection is limited. Negative results obtained by direct techniques do not rule out a ZIKV infection and require additional serological testing.

### 3.1.2 Indirect ZIKV Detection

Serology is the most commonly applied technique for diagnosing both recent and past ZIKV infections [48]. Serum samples obtained after the first week of clinical illness as well as samples that were tested negative or not tested by RT-PCR are most relevant for serological examination. If serology is negative in the first instance, particularly in the early phase of infection, antibody testing should be repeated on a serum sample taken between one and 2 weeks later. The detection of anti-ZIKV IgM, seroconversion, or a significant increase in the IgG titer in paired samples are indicative of an acute infection [48, 64, 67].

In primary ZIKV infections that often occur among travelers without previous flavivirus infection, anti-ZIKV IgM antibodies appear 4–7 days after disease onset, peak within 2 weeks, and remain detectable for approximately 12 weeks. Anti-ZIKV IgG antibodies appear shortly after IgM and are expected to persist lifelong (Fig. 3.1a, c) [4, 46, 81]. In contrast, most residents of ZIKV-endemic areas have preexisting antibodies against DENV or some other flavivirus (e.g., yellow fever virus) and contract ZIKV as a secondary flavivirus infection. According to our findings, these patients typically develop very high anti-ZIKV IgG titers early after the onset of symptoms, paralleled by a boost in IgG against the virus of the primary flavivirus infection, while the anti-ZIKV IgM response is often low or absent (Fig. 3.1b, d) [81]. Similar kinetics have been demonstrated for patients with secondary DENV infections, as described below. Considering the possibility of IgM negative results in the setting of secondary infections, the "IgM only" strategy, as currently recommended for ZIKV serological testing [60, 64], bears a high risk of misdiagnosis of active infections. Rather, additional IgG analysis is regarded mandatory [14, 75].

ZIKV-specific antibodies are most commonly analyzed by ELISA and IIFT, including in-house assays (e.g., CDC Zika MAC ELISA) and an increasing number of commercial tests. Immunochromatographic assays for point-of-care rapid diagnostic testing (RDT) are also available [95, 96] (Table 3.1). Most of these assays, however, are based on whole-virus antigen or virus glycoproteins and consequently suffer from extensive serological cross-reactivity with other flaviviruses (e.g., DENV, yellow fever, West Nile, and Japanese encephalitis virus), which leads to false-positive or uninterpretable data. Thus, cross-reactivity presents a major challenge for the interpretation of results and may preclude the identification of the specific infecting virus, especially in individuals previously infected with or vaccinated against a related flavivirus. Even when using plaque-reduction neutralization tests (PRNT) to confirm the diagnosis of ZIKV, as recommended in current testing algorithms, cross-neutralization cannot be excluded for secondarily-infected patients so that PRNT results may not always distinguish ZIKV from other flavivirus or multi-flavivirus infections [13, 17, 22, 46, 48, 69–71]. Therefore, efforts have been made in the development of widely applicable serological assays that provide high diagnostic sensitivity and specificity for ZIKV. So far, however, data reflecting assay performance is rare and has to be interpreted with caution as it depends on the validation cohort.

#### 3.1.2.1 ZIKV Differential Diagnosis Using IIFT Biochip Mosaics

IIFT biochip mosaics (Euroimmun, Lübeck, Germany) provide a suitable test platform for standardized multiparametric testing, and have been developed and manufactured in cooperation with the Robert Koch Institute (RKI, Berlin, Germany). Every test field contains a combination of biochips each of which is coated with another substrate (virus-infected cells). Thus, in a single analysis, a patient sample can be examined simultaneously for antibodies against all infectious agents relevant for differential diagnosis, i.e. identifying one specific pathogen amongst pathogens occurring in the same geographic

**Table 3.1** Available serological tests for the diagnosis of ZIKV infections (11/2016)

| Company | Assay* | Ig class | Format | References |
|---|---|---|---|---|
| ADI | RecombiVirus Zika Virus Envelop antibody ELISA[c] | IgM | ELISA | |
| | RecombiVirus Zika Virus Envelop antibody ELISA[c] | IgG | ELISA | |
| | RecombiVirus Zika Virus Envelop domain III ELISA[c] | IgM | ELISA | |
| | RecombiVirus Zika Virus Envelop domain III ELISA[c] | IgG | ELISA | |
| | RecombiVirus Zika Virus PrM antibody ELISA[c] | IgM | ELISA | |
| | RecombiVirus Zika Virus PrM antibody ELISA[c] | IgG | ELISA | |
| | RecombiVirus Zika Virus NS1 antibody ELISA[c] | IgM | ELISA | |
| | RecombiVirus Zika Virus NS1 antibody ELISA[c] | IgG | ELISA | |
| | RecombiVirus Zika Virus Capsid antibody ELISA[c] | IgM | ELISA | |
| | RecombiVirus Zika Virus Capsid antibody ELISA[c] | IgG | ELISA | |
| | RecombiVirus Zika Virus Env+NS1+Prm+Capsid antibody Combo ELISA[c] | IgM | ELISA | |
| | RecombiVirus Zika Virus Env+NS1+Prm+Capsid antibody Combo ELISA[c] | IgG | ELISA | |
| Artron | One Step Zika Virus Test Kit[a] | IgM/IgG | ICA (RDT) | |
| Biocan | Zika Virus IgG/IgM Antibody Test[a] | IgM/IgG | ICA (RDT) | |
| | Zika/Dengue/Chikungunya Combo Tests[a] | IgM/IgG | ICA (RDT) | |
| Chembio | DPP Zika IgM/IgG Assay[a] | IgM/IgG | ICA (RDT) | |
| CD | Zika Virus IgM ELISA | IgM | ELISA | |
| CDC | Zika MAC-ELISA[b] | IgM | ELISA | [23, 25] |
| CTK Biotech | RecombiLISA Zika IgM Test[d] | IgM | ELISA | |
| DA/CD | OneStep Zika Virus IgG/IgM RapiCard[c] | IgM/IgG | ICA (RDT) | |
| DIACHECK | DIACHECK Anti-ZIKA IgM[c] | IgM | ELISA | |
| | DIACHECK Anti-ZIKA IgG[c] | IgG | ELISA | |
| | DIACHECK Anti-ZIKA IgA[c] | IgA | ELISA | |
| Dia.Pro | ZIKA Virus IgM[a] | IgM | ELISA | |
| | ZIKA Virus IgG[a] | IgG | ELISA | |
| | Zika Virus IgG Avidity[a] | IgG | ELISA | |
| DRG | Zika Virus IgM μ-capture ELISA | IgM | ELISA | |

(continued)

**Table 3.1** (continued)

| Company | Assay* | Ig class | Format | References |
|---|---|---|---|---|
| Euroimmun | Anti-Zika virus IIFT[a] | IgM/IgG | IIFT | |
| | Arbovirus Fever Mosaic 2 IIFT[a] | IgM/IgG | IIFT | |
| | Arbovirus Profile 3 IIFT[a] | IgM/IgG | IIFT | |
| | Anti-Zika virus ELISA (IgM)[a] | IgM | ELISA | [2, 14, 34, 35, 38, 41, 55, 72, 81, 86, 100] |
| | Anti-Zika virus ELISA (IgG)[a] | IgG | ELISA | [41, 81, 86, 100] |
| IBL | Zika Virus IgM μ-capture ELISA[a] | IgM | ELISA | |
| InBios | ZIKV Detect IgM Capture ELISA[b] | IgM | ELISA | |
| LumiQuick | QuickProfile Zika Virus IgG/IgM Combo Test[a] | IgM/IgG | ICA (RDT) | |
| Mikrogen | alphaWell Zika Virus IgM μ-capture | IgM | ELISA | |
| | recomLine Tropical Fever IgM[a] | IgM | LIB | |
| | recomLine Tropical Fever IgG[a] | IgG | LIB | |
| MyBioSource | Qualitative Human Zika Virus IgM (ZV-IgM) ELISA[c] | IgM | ELISA | |
| | Qualitative Human Zika Virus IgG (ZV-IgG) ELISA[c] | IgG | ELISA | |
| NovaTec | NovaLisa Zika Virus IgM μ-capture ELISA[a] | IgM | ELISA | |
| R-Biopharm | RIDASCREEN Zika Virus IgM μ-capture | IgM | ELISA | |
| SD Biosensor | STANDARD E Zika IgM ELISA | IgM | ELISA | |
| | STANDARD Q Zika IgM/IgG | IgM/IgG | ICA (RDT) | |
| Viramed | Zika Virus ViraStripe IgM[a] | IgM | LIB | |
| | Zika Virus ViraStripe IgG[a] | IgG | LIB | |
| Vircell | ZIKV-DENV-CHIKV IFA IgM[a] | IgM | IIFT | |
| | ZIKV-DENV-CHIKV IFA IgG[a] | IgG | IIFT | |
| | ZIKA ELISA IgM[d] | IgM | ELISA | |
| | ZIKA ELISA IgG[d] | IgG | ELISA | |
| | ZIKA VIRCLIA IgM MONOTEST[d] | IgM | CLIA | |
| | ZIKA VIRCLIA IgG MONOTEST[d] | IgG | CLIA | |
| Viro-Immun | VIR-ELISA Zika Virus[c] | IgM | ELISA | |
| | VIR-ELISA Zika Virus[c] | IgG | ELISA | |
| | VIR-ELISA Zika Virus[c] | IgA | ELISA | |

*CLIA* chemiluminescence assay, *ELISA* enzyme-linked immunosorbent assay, *ICA* immunochromatographic assay, *IIFT* indirect immunofluorescence test, *LIB* line immunoblot, *RDT* rapid diagnostic test

*Regulatory status (if available) according to suppliers' official websites and test instructions

[a]CE/IVD certified

[b]Authorized by FDA under an Emergency Use Authorization for use by authorized laboratories only [32]

[c]For research use only

[d]Coming soon

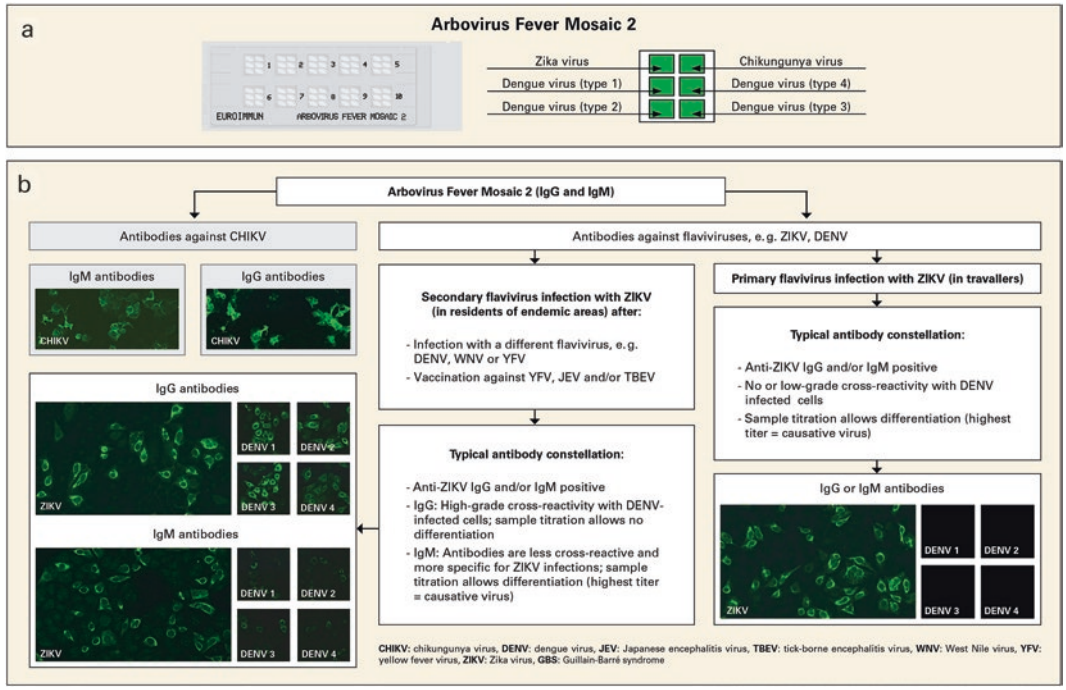


**Fig. 3.2** Arbovirus Fever Mosaic 2 (Euroimmun) for the detection of antibodies against ZIKV, DENV and CHIKV by indirect immunofluorescence. (**a**) Microscope slide with ten analysis fields each containing six biochips coated with differently infected cells (ZIKV, DENV 1, DENV 2, DENV 3, DENV 4, CHIKV). (**b**) Flow chart for the differential diagnosis of arbovirus infections, focusing on the characteristic antibody constellations and reactivity patterns in primary and secondary ZIKV infections

region and causing similar clinical symptoms. Further advantages as compared to in-house IIFTs are high-level standardization for increased reproducibility among patients and laboratories, rapid performance, storability, and inclusion of uninfected control cells for the recognition of unspecific reactivity.

The **Euroimmun Arbovirus Fever Mosaic 2 IIFT** consists of a combination of six cell substrates infected with ZIKV, CHIKV or DENV serotypes 1–4 (Fig. 3.2a). The mosaic's diagnostic performance is indicated in Table 3.2, including assay sensitivity in detecting confirmed infections with ZIKV, DENV or CHIKV, as well as assay specificity among healthy blood donors from non-endemic areas. The combination of antigenically related viruses on adjacent biochips also enables the investigation of potentially cross-reactive antibodies: Whilst there is generally no or only low-grade cross-reactivity in a primary flavivirus infection, high grade cross-reactivity is typical for secondary infections (e.g., a ZIKV infection following a DENV infection), usually being stronger for IgG than for IgM antibodies. Titration of the patient sample on the mosaic may enable the determination of a dominant end-point titer for the virus causing the infection (Fig. 3.2b). If IIFT does not allow unambiguous differentiation, an ELISA-based approach is appropriate (Fig. 3.3).

#### 3.1.2.2 Detection of Anti-ZIKV IgM and IgG Using a Highly Specific NS1-Based ELISA

In order to overcome the problem of serological cross-reactivity associated with whole virus-based assays, recombinant proteins have recently been given priority in the development of more efficient antigenic substrates for ZIKV serodiagnosis. Since the non-structural protein 1 (NS1)

**Table 3.2** Sensitivity and specificity of the Arbovirus Fever Mosaic 2 IIFT (Euroimmun)

| Substrate | Ig class | Sensitivity | | Specificity | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| Zika virus | IgM | 97[a-c] | 96.9 | 211[a,i,k] | 98.1 |
| | IgG | 104[a-c] | 96.8 | 258[a,i,k] | 93.4 |
| Dengue virus (types 1–4) | IgM | 65[a,d,e] | 98.5 | 184[e,i] | 96.2 |
| | IgG | 59[a,d,e] | 96.6 | 251[e,i] | 96.4 |
| Chikungunya virus | IgM | 174[a,f-h] | 97.7 | 256[g-i] | 99.6 |
| | IgG | 117[a,f,g,i] | 95.7 | 200[a,i] | 98.5 |

[a]Samples pre-characterization using in-house methods at the WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research (WHOCC, Hamburg, Germany)
[b]Samples from ZIKV-RT-PCR-confirmed patients from the Dominican Republic (BocaBiolistics, USA)
[c]Samples from ZIKV-RT-PCR-confirmed German and Italian patients
[d]Samples pre-characterization using in-house methods at the Robert Koch Institute (RKI, Berlin, Germany)
[e]Samples pre-characterization using Dengue IgM Capture ELISA or Dengue IgG Capture ELISA (Panbio Diagnostics, Brisbane, Australia) at the University Jedda (Saudi Arabia)
[f]Samples pre-characterization using in-house anti-CHIKV ELISA at the Centre National de Référence des Arbovirus (Marseille, France)
[g]Samples pre-characterization using in-house anti-CHIKV ELISA at Cerba Specimen Services (France)
[h]Samples pre-characterization using in-house CHIKV MAC-ELISA at the Centers of Disease Control and Prevention (CDC) Arboviral Diseases Branch (Fort Collins, Colorado, USA) [43]
[i]Samples from healthy blood donors provided by the University Medical Center Schleswig-Holstein (Lübeck, Germany)
[k]Samples from healthy pregnant women provided by Laboratory Schottdorf (Augsburg, Germany)

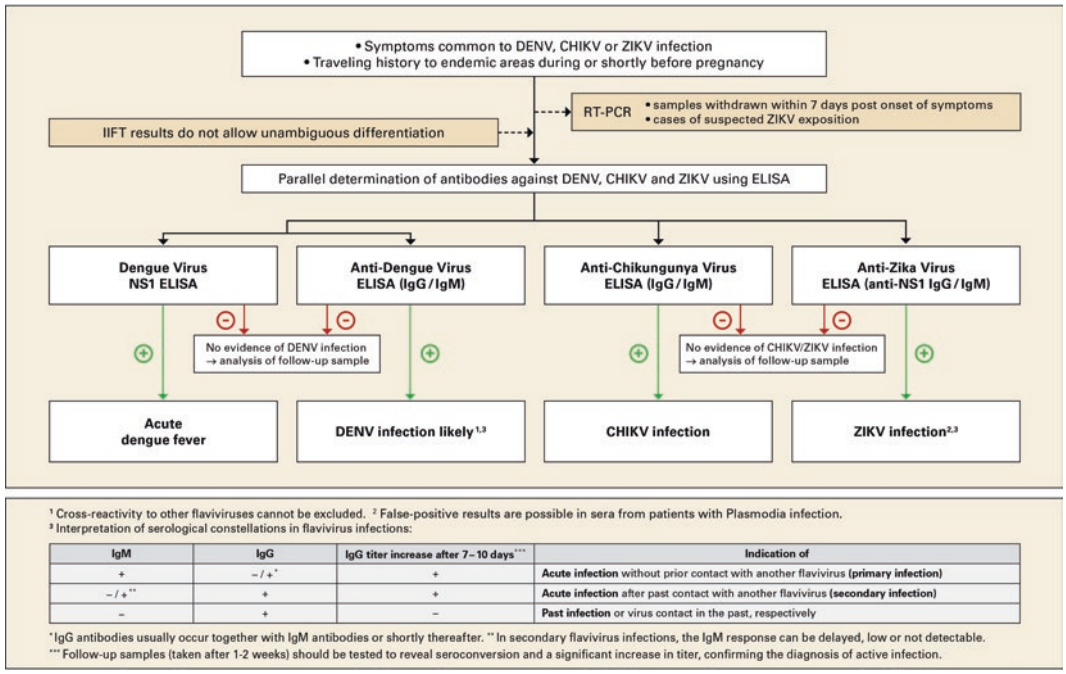


**Fig. 3.3** Algorithm for the serological differential diagnosis in suspected cases of DENV, CHIKV or ZIKV infections using Euroimmun ELISA. IgG and IgM antibodies against DENV, CHIKV and ZIKV should be determined in parallel

**Table 3.3** Sensitivity of the NS1-based Anti-Zika Virus ELISA (Euroimmun)

| Cohort | Origin of ZIKV infection | n | Anti-ZIKV ELISA sensitivity (CI 95%)[c] | | | References |
|---|---|---|---|---|---|---|
| | | | IgM | IgG | IgM/IgG | |
| Travelers returning from ZIKV-endemic areas (primary ZIKV infection) | | | | | | |
| RT-PCR-confirmed ZIKV infection[a] | Haiti or N/A | 5 | 100% (51.1–100%) | 60.0% (22.9–88.4%) | 100% (51.1–100%) | [81] |
| IIFT-confirmed ZIKV infection[b] | Brazil, Colombia or N/A | 26 | 80.8% (61.7–92.0%) | 69.2% (49.9–83.7%) | 96.2% (79.6–100%) | [81] |
| Residents in ZIKV-endemic areas (secondary ZIKV infection) | | | | | | |
| RT-PCR-confirmed ZIKV infection[a] | Suriname, Colombia, Dominican Republic | 12 | 41.7% (19.3–68.1%) | 100% (71.8–100%) | 100% (71.8–100%) | [81] |
| IIFT-confirmed ZIKV infection[a] | Colombia | 38 | 7.9 % (2.0–21.5%) | 89.5% (75.3–96.4%) | 89.5% (75.3–96.4%) | [81] |
| IIFT-confirmed acute ZIKV infection | Brazil | 10 | 100% (67.9–100%) | 50% (23.7–76.3%) | 100% (67.9–100%) | [41] |

*CI* confidence interval, *ELISA* enzyme-linked immunosorbent assay, *IIFT* indirect immunofluorescence test, *N/A* not available, *RT-PCR* reverse transcription-polymerase chain reaction, *ZIKV* Zika virus
[a]Samples taken from different patients ≥6 days post symptom onset
[b]Samples taken from different patients ≥1 days post symptom onset (no data available for ≥6 days)
[c]Cut-off ratio for positivity: ≥1.1

had been shown to be a suitable candidate [21], the **Euroimmun Anti-Zika Virus ELISA** was developed based on recombinant ZIKV NS1 as solid-phase antigen [81]. Meanwhile, this ELISA has become widely established in prenatal and routine diagnostic settings [2, 14, 34, 35, 55, 72, 100] (Fig. 3.3). Its diagnostic performance was evaluated in cooperation with renowned European institutes for tropical and travel medicine using sera from residents in ZIKV-endemic areas, returning travelers, individuals with potentially cross-reactive antibodies, and blood donors of different age groups and origin [41, 81, 86]. Highest ELISA sensitivity (100% in RT-PCR-confirmed ZIKV infections) was achieved by combined IgM/IgG testing (Table 3.3). In cohorts of healthy individuals, ELISA specificity amounted to ≥99.0% for IgM and 96.7–100% for IgG (Table 3.4). Referring to potentially cross-reactive samples, specificity was 97.1–100% for IgM and 96.0–100% for IgG. Positivity in very few samples from patients infected with another flavivirus (WNV, JEV) may be due to co-infection with ZIKV (true-positive results) or due to cross-reactivity (false-positive results). Importantly, ELISA reactivity was neither observed in DENV-infected patients from ZIKV non-endemic regions nor in individuals with high-titer anti-DENV antibodies (including secondary infections), irrespective of the DENV serotype [38, 81] (Table 3.4). Furthermore, Granger *et al*. reported an excellent overall agreement of the Euroimmun Anti-Zika Virus IgM ELISA with the CDC Zika MAC-ELISA (100% correlation) [38].

Analysis of potential interfering factors (polyclonal B-cell stimulation triggered by infections with Epstein-Barr virus, Mycoplasma pneumoniae, cytomegalovirus, human immunodeficiency virus or Plasmodium spp.; rheumatoid factor; autoimmune antibodies) revealed false-positive anti-ZIKV IgM/IgG results in about 20–30% of patients with acute and <2% in those with past Plasmodium infection. Among all other factors, interferences were observed in ≤6% for IgM and 0% for IgG [41, 81, 86] [and unpublished data].

**Table 3.4** Specificity of the NS1-based Anti-Zika Virus ELISA (Euroimmun)

| Cohort | Anti-ZIKV ELISA IgM | | Anti-ZIKV ELISA IgG | | References[e] |
|---|---|---|---|---|---|
| | n | Specificity (CI 95%)[c] | n | Specificity (CI 95%)[c] | |
| Healthy control samples | | | | | |
| German pregnant women | 100 | 100% (95.6–100%) | 100 | 100% (95.6–100%) | [81] |
| Healthy prepartal women[b] | 20 | 100% (83.2–100%) | 20 | 100% (83.2–100%) | [38] |
| Zimbabwean blood donors | 128 | 100% (96.5–100%) | 128 | 100% (96.5–100%) | [81] |
| Argentinian blood donors | 99 | 99.0% (94.0–100%) | 99 | 100% (95.5–100%) | [81] |
| US-American blood donors | 100 | 100% (95.6–100%) | 100 | 99.0% (94.0–100%) | [81] |
| German blood donors | 500 | 99.8% (98.8–100%) | 500 | 99.8% (98.8–100%) | [81] |
| Healthy blood donors[b] | 30 | 100% (86.5–100%) | 30 | 96.7% (81.9–100%) | [38] |
| German children | 88 | 100% (95.0–100%) | 88 | 100% (95.0–100%) | [81] |
| Potentially cross-reactive samples | | | | | |
| DENV[a] infection (high median anti-DENV IgM) | 47 | 100% (91.0–100%) | 47 | 100% (91.0–100%) | [81] |
| DENV[a] infection (high median anti-DENV IgG) | 46 | 100% (90.8–100%) | 46 | 100% (90.8–100%) | [81] |
| DENV[a] infection | 16 | 100% (77.3–100%) | 10 | 100% (67.9–100%) | [41] |
| Early convalescent DENV[a] infection in individuals from a ZIKV non-endemic region | 7 | 100% (59.6–100%) | 7 | 100% (59.6–100%) | [38] |
| Suspected secondary DENV[a] infection in individuals from a ZIKV non-endemic region | 13 | 100% (73.4–100%) | 13 | 100% (73.4–100%) | [38] |
| DENV (type 1) infection | 3 | 100% (29.2–100%) | 3 | 100% (29.2–100%) | [86] |
| DENV (type 1) infection | 8 | 100% (62.8–100%) | 8 | 100% (62.8–100%) | UD |
| DENV (type 2) infection | 4 | 100% (45.4–100%) | 4 | 100% (45.4–100%) | [86] |
| DENV (type 2) infection | 10 | 100% (67.9–100%) | 10 | 100% (67.9–100%) | UD |
| DENV (type 3) infection | 2 | 100% (29.0–100%) | 2 | 100% (29.0–100%) | [86] |
| DENV (type 3) infection | 5 | 100% (51.1–100%) | 5 | 100% (51.1–100%) | UD |
| DENV (type 4) infection | 1 | 100% (16.8–100%) | 1 | 100% (16.8–100%) | [86] |
| DENV (type 4) infection | 3 | 100% (38.3–100%) | 3 | 100% (38.3–100%) | UD |
| YFV vaccination | 12 | 100% (71.8–100%) | 12 | 100% (71.8–100%) | [81] |
| YFV vaccination | 15 | 100% (76.1–100%) | 15 | 100% (76.1–100%) | [41] |
| YFV vaccination | 10 | 100% (67.9–100%) | 10 | 100% (67.9–100%) | [86] |
| WNV infection | 34 | 97.1% (83.8–100%)[d] | 34 | 100% (87.9–100%) | [81] |
| WNV infection | 10 | 100% (67.9–100%) | 13 | 100% (73.4–100%) | [38] |
| JEV infection | 25 | 100% (84.2–100%) | 25 | 96.0% (78.9–100%)[d] | [81] |
| CHIKV infection | 19 | 100% (80.2–100%) | 19 | 100% (80.2–100%) | [81] |
| CHIKV infection | 4 | 100% (45.4–100%) | 5 | 100% (51.1–100%) | [38] |
| SLEV infection | 2 | 100% (29.0–100%) | 6 | 100% (54.1–100%) | [38] |
| TBEV infection | 38 | 100% (89.1–100%) | 21 | 100% (81.8–100%) | [41] |
| TBEV vaccination | | N/A | 52 | 100% (91.8–100%) | [41] |
| HCV infection | | N/A | 16 | 100% (77.3–100%) | [41] |

*CHIKV*, chikungunya virus, *CI* confidence interval, *DENV* dengue virus, *ELISA* enzyme-linked immunosorbent assay, *HCV* hepatitis C virus, *JEV* Japanese encephalitis virus, *N/A* not available, *SLEV* St. Louis encephalitis virus, *TBEV* tick-borne encephalitis virus, *UD* unpublished data, *WNV* West Nile virus, *YFV* yellow fever virus, *ZIKV* Zika virus

[a]DENV serotype not known

[b]Origin of sample donors not available

[c]Cut-off ratio for positivity: ≥1.1

[d]Anti-ZIKV reactivity may be due to ZIKV/WNV or ZIKV/JEV co-infections (true-positive results) or due to cross-reactivity (false-positive results)

[e]Unpublished data (UP) were provided by K. Steinhagen

## 3.2 Laboratory Testing for DENV Infections

Any of the four DENV serotypes can cause asymptomatic infections, dengue fever (DF) or dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Importantly, a secondary infection by a heterologous DENV serotype is associated with a high risk for DHF/DSS due to immune enhancement [10, 79]. Diagnostics can be performed through isolation of the virus, detection of viral RNA or antigen, detection of specific antibodies, or (optimally) a combination thereof [27, 39, 64, 66, 82, 84]. Evidence of the value of combining DENV antigen- and antibody-based test results has been comprehensively described in previous publications [8, 36, 61].

### 3.2.1 Direct DENV Detection

Direct methods, including virus isolation and detection of viral RNA by RT-PCR are most efficient during the first 4–5 days after the onset of symptoms [18, 20, 45, 47, 74]. RT-PCR is both sensitive and specific with respect to DENV confirmation and serotyping, therefore representing the method of choice for the examination of patient samples obtained in the early acute stage of illness [15, 63]. However, RT-PCR requires specific reagents and laboratory equipment as well as trained personnel, while virus isolation is time consuming. Due to the short viremic phase, RT-PCR may already be negative by the time a patient consults a doctor.

Alternatively, many standardized commercial assays for the detection of DENV antigens, particularly NS1, have been developed [3, 29, 30, 61, 65]. NS1 is detectable at the onset of symptoms in both primary and secondary DENV infections and remains detectable past the viremic phase (up to 9 days from disease onset), thus offering a larger time frame for diagnosis than other direct techniques. In addition, high NS1 levels correlate with the development of DHF [1, 29, 52, 99]. Importantly, sera from patients with acute ZIKV infection do not appear to cross-react with DENV NS1 antigen tests, indicating high specificity [54]. Rapid diagnostic tests (RDT) for DENV NS1 detection provide opportunities for point-of-care diagnosis, but should be interpreted with caution since their sensitivity is inherently limited and inferior to ELISA testing [5, 42, 65]. Negative results obtained by direct methods do not rule out a DENV infection and require additional serological testing.

#### 3.2.1.1 Detection of DENV NS1 Antigen Using ELISA

The **Euroimmun Dengue Virus NS1 ELISA** is coated with monoclonal anti-DENV NS1 antibodies that specifically bind NS1 of all four DENV serotypes and enable highly sensitive antigen detection. Analysis of 35 clinically and serologically pre-characterized sera provided by the INSTAND quality assessment scheme (2010 to 2016) revealed 100% agreement between the INSTAND target values and the results obtained using the Euroimmun Dengue Virus NS1 ELISA, indicating 100% sensitivity and specificity. Parallel investigation of specific antibodies against DENV, ZIKV and CHIKV is recommended considering the relevance of early diagnosis and the possibility of co-infections (Fig. 3.3).

### 3.2.2 Indirect DENV Detection

Serology is frequently used for routine diagnosis of DENV infections and preferentially applied after the time slot for direct virus detection (>3–4 days post symptom onset) or subsequent to negative testing by direct methods [39]. In primary DENV infections, specific IgM antibodies are detectable in 50% of patients by the third day after symptom onset, increasing to 80–99% by day five to ten. IgM levels peak after approximately 2 weeks of illness and decline to undetectable levels after 2–3 months. Anti-DENV IgG starts to increase at the end of the first week of illness and remains detectable for months and years [39, 63, 84]. In contrast, during secondary infection, the IgM response is often delayed, low or undetectable, while IgG titers rise rapidly within the first 2 days after symptom onset, the

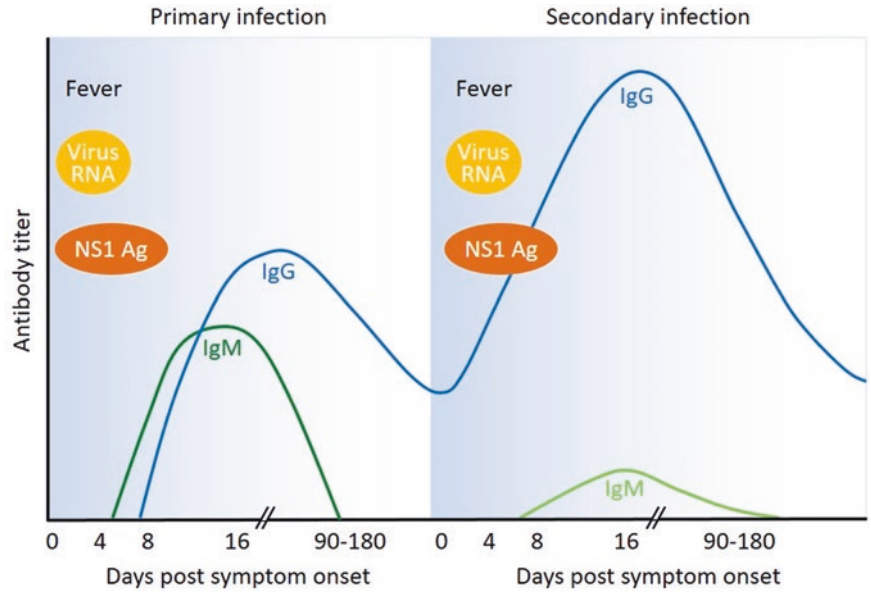


**Fig. 3.4** Time course of serological parameters in primary and secondary DENV infection. In secondary DENV infections, the IgM response is variable, and in some cases undetectable

latter showing high cross-reactivity with other flaviviruses (Fig. 3.4) [19, 39, 75, 77, 87].

Acute DENV infections are indicated serologically by the presence of specific IgM, seroconversion or a fourfold or greater increase in IgG titer. Since the delay or absence of detectable IgM levels in some secondary infections may lead to misdiagnosis, the combined testing of IgM and IgG has been proposed as an effective strategy [75, 76]. As DENV-specific antibodies of class IgA are produced in parallel to IgM in about 70% of cases, the additional determination of IgA can assure the diagnosis of a recent primary infections, especially when a follow-up sample is not yet available or if the IgG response is not yet detectable [57]. For the classification of primary and secondary DENV infections, some protocols use IgM/IgG ratios, with capture ELISAs being the most common assays for this purpose [26, 31, 78, 84]. Alternatively, a hemagglutination inhibition (HI) titer exceeding 1:1280 in convalescent serum is considered indicative of a secondary DENV infection [84].

A large number of commercial assays for DENV serological testing with variable degrees of sensitivity and specificity are currently available, with ELISA, IIFT and immunochromatographic RDTs representing preferred methods for rapid, simple and high-throughput testing (Table 3.5) [5, 8, 40, 83, 84]. HI assays and PRNTs are further options, but not routinely used because they are not standardized, time consuming, labor intensive, low throughput and restricted to Biosafety Level 3. Noteworthy, the performance of anti-DENV RDTs appears problematic [5]. This was also reflected by the INSTAND DENV proficiency testing in 2016, demonstrating average pass rates for anti-DENV IgG positive samples of 8.3–70.8% by laboratories using rapid tests compared to 97.7–100% using ELISA and 90.0–100% using IIFT [42]. In a WHO multicenter study, anti-DENV IgM RDTs showed mean sensitivities ranging from 20.5% to 97.7% and specificities between 76.6% and 90.6%, while IgM ELISA kits had higher sensitivities of 61.5–99.0% and specificities of 79.9–97.8% [40].

**Table 3.5** Available serological tests for the diagnosis of DENV infections (11/2016)

| Company | Assay | Ig class | Format | References [5, 80, 83] |
|---|---|---|---|---|
| Access Bio | CareStart Dengue Combo (NS1+IgM/IgG) | IgM/IgG | ICA (RDT) | |
| ADI | Human Anti-Dengue Virus IgM ELISA | IgM | ELISA | |
| | Human Anti-Dengue Virus IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 1 Envelop protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 2 Envelop protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 3 Envelop protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 4 Envelop protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 1 prM protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 2 prM protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 3 prM protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 4 prM protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 1+2+3+4 Envelop protein IgG ELISA | IgG | ELISA | |
| | Human Anti-Dengue virus 1+2+3+4 prM protein IgG ELISA | IgG | ELISA | |
| Artron | Dengue Virus IgG/IgM Antibody | IgM/IgG | ICA (RDT) | |
| | Dengue IgG/IgM/NS1 Combo | IgM/IgG | ICA (RDT) | |
| Bio-Rad | Platelia Dengue IgA Capture | IgA | ELISA | [26] |
| | Platelia Dengue IgG Capture | IgG | ELISA | |
| | RDT Dengue IgA/IgG | IgA/IgG | ICA (RDT) | |
| Biocan | Dengue IgG/IgM Antibody | IgM/IgG | ICA (RDT) | |
| | Dengue IgA/IgG/IgM Antibody (Triplex) | IgM/IgG/IgA | ICA (RDT) | |
| | Dengue IgA/IgG/IgM Antibody & NS1 (Fourplex) | IgM/IgG/IgA | ICA (RDT) | |
| | Zika/Dengue/Chikungunya Combo Tests | IgM/IgG | ICA (RDT) | |
| Biosynex | Immunoquick Dengue Fever IgG and IgM | IgM/IgG | ICA (RDT) | [7] |
| Calbiotech | Dengue Virus IgM ELISA | IgM | ELISA | |
| | Dengue Virus IgG ELISA | IgG | ELISA | |
| CD | Dengue IgM Capture ELISA | IgM | ELISA | |
| | Dengue IgG ELISA | IgG | ELISA | |
| | Dengue Virus IgG Human ELISA | IgG | ELISA | |
| | Dengue Fever IgG-IgM (S/WB/P) Rapid Test (Cassette) | IgM/IgG | ICA (RDT) | |
| Core | Core Dengue (IgG+IgM ) | IgM/IgG | ICA (RDT) | [6] |

(continued)

**Table 3.5** (continued)

| Company | Assay | Ig class | Format | References [5, 80, 83] |
|---|---|---|---|---|
| CTK Biotech | RecombiLISA Dengue IgM Test | IgM | ELISA | |
| | RecombiLISA Dengue IgG Test | IgM | ELISA | |
| | OnSite Dengue IgG/IgM Combo Rapid Test | IgM, IgG | ICA (RDT) | [8] |
| | OnSite Duo Dengue Ag-IgG/IgM Rapid Test | IgM, IgG | ICA (RDT) | |
| | OnSite Duo Dengue IgG/IgM-CHIK IgM Rapid Test | IgM | ICA (RDT) | |
| DA/CD | AccuDiag Dengue IgM ELISA | IgM | ELISA | |
| | AccuDiag Dengue IgG ELISA | IgG | ELISA | |
| | Dengue IgG/IgM | IgM/IgG | ELISA | |
| | OneStep Dengue NS1 Antigen IgG/IgM Antibody Duo Panel RapidCard | IgM/IgG | ICA (RDT) | |
| | OneStep Dengue Fever IgG/IgM RapiCard | IgM/IgG | ICA (RDT) | |
| Dia.Pro | DENM – IgM | IgM | ELISA | |
| | DENG – IgG | IgG | ELISA | |
| DiaSorin | Dengue IgM | IgM | ELISA | |
| | Dengue IgG | IgG | ELISA | |
| | Dengue Virus IgM μ-capture | IgM | ELISA | |
| DRG | Dengue (1–4) IgM capture ELISA | IgM | ELISA | |
| | DengueVirus IgM ELISA | IgM | ELISA | [42] |
| | Dengue Virus IgG ELISA | IgG | ELISA | [42] |
| | Dengue IgG/IgM Cassette Test | IgM/IgG | ICA (RDT) | |
| | Dengue Rapid Test | IgM/IgG | ICA (RDT) | |
| Euroimmun | Mosaic: Dengue virus types 1–4 (IgG or IgM) | IgM/IgG | IIFT | [42] |
| | Flavivirus Profile 2 (IgG or IgM) | IgM/IgG | IIFT | |
| | Flavivirus Profile 3 (IgG or IgM) | IgM/IgG | IIFT | |
| | Arbovirus Fever Mosaic 1 (IgG or IgM) | IgM/IgG | IIFT | |
| | Arbovirus Fever Mosaic 2 (IgG or IgM) | IgM/IgG | IIFT | |
| | Arbovirus Profile 3 (IgG or IgM) | IgM/IgG | IIFT | |
| | Anti-Dengue virus ELISA (IgM) | IgM | ELISA | [42] |
| | Anti-Dengue virus ELISA (IgG) | IgG | ELISA | [42] |
| Focus | Dengue Virus IgM Capture DxSelect | IgM | ELISA | [40, 42] |
| | Dengue Virus IgG DxSelect | IgG | ELISA | [42] |
| | Dengue Dx IgG/IgM Rapid Test | IgM/IgG | ICA (RDT) | [42] |
| GenWay | Dengue Virus IgM-ELISA | IgM | ELISA | |
| | Dengue Virus IgG-ELISA | IgG | ELISA | |
| IBL | Dengue Virus IgM μ-capture ELISA | IgM | ELISA | |
| | Dengue Virus IgM ELISA | IgM | ELISA | [42] |
| | Dengue Virus IgG ELISA | IgG | ELISA | [42] |
| InBios | DENV Detect IgM Capture ELISA | IgM | ELISA | [94] |
| | DENV Detect IgG ELISA | IgG | ELISA | |

(continued)

**Table 3.5** (continued)

| Company | Assay | Ig class | Format | References [5, 80, 83] |
|---|---|---|---|---|
| J. Mitra & Co | Dengue IgM Microlisa | IgM | ELISA | |
| | Dengue IgG Microlisa | IgG | ELISA | |
| | Dengue Day1 Test | IgM, IgG | ICA (RDT) | |
| | Diagnos Dengue Card | IgM, IgG | ICA (RDT) | |
| LumiQuick | QuickProfile Dengue IgG/IgM Combo Test | IgM/IgG | ICA (RDT) | |
| | QuickProfile Dengue NS1 Antigen & IgG/IgM Duo Panel | IgM/IgG | ICA (RDT) | |
| MIROGEN | alphaWell Dengue IgM μ-capture | IgM | ELISA | |
| | alphaWell Dengue IgM | IgM | ELISA | |
| | alphaWell Dengue IgG | IgG | ELISA | |
| | recomLine Tropical Fever IgM | IgM | LIB | |
| | recomLine Tropical Fever IgG | IgG | LIB | |
| MP Diagnostics | Dengue Virus IgG ELISA | IgG | ELISA | |
| MyBioSource | Dengue Virus IgM ELISA | IgM | ELISA | |
| | Dengue Virus IgG ELISA | IgG | ELISA | |
| NovaTec | NovaLisa Dengue Virus IgM μ-capture ELISA | IgM | ELISA | |
| | NovaLisa Dengue Virus IgM ELISA | IgM | ELISA | [42] |
| | NovaLisa Dengue Virus IgG ELISA | IgG | ELISA | |
| Omega | Pathozyme-Dengue M Capture | IgM | ELISA | [40, 59] |
| | Pathozyme-Dengue G | IgG | ELISA | |
| | Visitect Dengue | IgM/IgG | ICA (RDT) | |
| OrangeLife | OL Dengue IgG/IgM | IgM/IgG | ICA (RDT) | |
| | OL Combo Dengue NS1/IgG/IgM | IgM/IgG | ICA (RDT) | |
| Orgenics/ Alere | ImmunoComb II Dengue IgM & IgG BiSpot | IgM/IgG | EIA | [8, 73] |
| Panbio/Alere | Dengue IgM Capture ELISA | IgM | ELISA | [8, 26, 40, 42, 59, 89] |
| | Dengue IgG Capture ELISA | IgG | ELISA | [8, 26, 89] |
| | Dengue IgG Indirect ELISA | IgG | ELISA | [42] |
| | Dengue Duo IgM and IgG Capture ELISA | IgM/IgG | ELISA | [24, 73, 76, 88] |
| | Japanese Encephalitis – Dengue IgM Combo ELISA | IgM | ELISA | [51] |
| | Dengue Duo Cassette | IgM/IgG | ICA (RDT) | [7, 36, 40, 42, 59, 80] |
| PROGEN | Dengue Virus Type 2 Antibody detection kit | IgM/IgG | IIFT | [42] |
| R-Biopharm | RIDASCREEN Dengue Virus IgM | IgM | ELISA | |
| | RIDASCREEN Dengue Virus IgG | IgG | ELISA | |
| SD Standard/ Alere | SD Dengue IgM Capture ELISA | IgM | ELISA | [8, 40] |
| | SD Dengue IgG Capture ELISA | IgG | ELISA | [8] |
| | SD BIOLINE Dengue Duo (Dengue NS1 Ag + IgG/IgM) | IgM/IgG | ICA (RDT) | [7, 8, 42, 61, 85, 90, 93] |
| | SD BIOLINE Dengue IgG/IgM | IgM/IgG | ICA (RDT) | [6, 40, 42] |
| | SD BIOLINE Dengue IgG/IgM WB | IgM/IgG | ICA (RDT) | |

(continued)

**Table 3.5** (continued)

| Company | Assay | Ig class | Format | References [5, 80, 83] |
|---|---|---|---|---|
| SD Biosensor | STANDARD E Dengue IgM ELISA | IgM | ELISA | |
| | STANDARD E Dengue IgG ELISA | IgG | ELISA | |
| | STANDARD Q Dengue IgM/IgG | IgM/IgG | ICA (RDT) | |
| | STANDARD Q Dengue Duo | IgM/IgG | ICA (RDT) | |
| Virion\Serion | SERION ELISA classic Dengue Virus IgM | IgM | ELISA | |
| | SERION ELISA classic Dengue Virus IgG | IgG | ELISA | |
| Vircell | ZIKV-DENV-CHIKV IFA IgM | IgM | IIFT | |
| | ZIKV-DENV-CHIKV IFA IgG | IgG | IIFT | |
| | DENGUE ELISA IgM CAPTURE | IgM | ELISA | |
| | DENGUE ELISA IgG | IgG | ELISA | |
| | DENGUE VIRCLIA IgM MONOTEST | IgM | CLIA | |
| | DENGUE VIRCLIA IgG MONOTEST | IgG | CLIA | |
| Zephyr | Denguecheck Combo | IgM/IgG | ICA (RDT) | [40] |

*CLIA* chemiluminescence assay, *ELISA* enzyme-linked immunosorbent assay, *ICA* immunochromatographic assay, *IIFT* indirect immunofluorescence test, *LIB* line immunoblot, *RDT* rapid diagnostic test

Since cross-reactivity between the DENV serotypes and within the flaviviruses has been reported as a major limitation of DENV serological assays, differential diagnosis with respect to all four DENV serotypes and other co-endemic viruses causing similar clinical manifestations (e.g., ZIKV, CHIKV, yellow fever, Japanese encephalitis virus,) is of particular importance, just like the determination of the pathogenic agent using direct techniques [6, 8, 27] .

#### 3.2.2.1 DENV Serotyping and Differential Diagnosis Using IIFT Biochip Mosaics

In the **Euroimmun Anti-Dengue Virus IIFT**, every test field contains four biochips, each coated with cells infected with one of the four DENV serotypes. This enables the simultaneous examination of a patient sample for reactivity (IgM or IgG) against DENV types 1–4 and in some cases serotyping by endpoint titration. Other Euroimmun IIFT biochip mosaics (e.g., Flavivirus Profiles, Arbovirus Profiles and Arbovirus Fever Mosaics) combine DENV-infected cells with further flavivirus substrates (Fig. 3.2, Tables 3.2 and 3.5). They are helpful for differential diagnosis and in consideration of potential cross-reactions with related viruses, representing a fast and simple alternative to more elaborate methods.

#### 3.2.2.2 Detection of Anti-DENV Antibodies Using ELISA

The **Euroimmun Anti-Dengue Virus ELISA** is based on highly purified virus particles of serotype 2. Because of the structural similarity between DENV 1 to 4, use of a single serotype is sufficient for the reliable detection of antibodies (IgM or IgG) against any of the virus types. In clinically characterized sera the IgM and IgG ELISA demonstrated 100% sensitivity and 99% specificity. The correlation between the Euroimmun Anti-Dengue Virus ELISA and the PANBIO Dengue Capture ELISA amounted to 97% for IgM and 99% for IgG (unpublished data). Due to use of whole-virus antigen, cross-reactions with other flavivirus antibodies, however, cannot be excluded (Fig. 3.3).

## 3.3 Serological Testing for CHIKV Infections

The confirmation of CHIKV infection by laboratory diagnostic means is analogous to ZIKV and DENV infections, as described above. In brief, during the first 5 days of infection, the identification of CHIKV is most sensitive using RNA detection (RT-PCR) or viral culture. Thereafter, specific antibodies against CHIKV are reliable indicators of disease, and can be detected from about 3–5 days after clinical onset [12, 50, 62, 92]. The determination of specific IgM and IgG can be performed using HI, virus neutralization and also by ELISA or IIFT which are preferable tests in the routine diagnostic settings. In addition, rapid point-of-care assays are available but suffer from poor performance [11, 43]. Table 3.6 presents current commercial assays for CHIKV serology. Differential diagnosis should include other co-circulating infections causing similar symptoms (e.g., dengue fever, Fig. 3.3) and should take into account cross-reactivity within the alphavirus genus. Reliable interpretation of test results and differential diagnosis can be achieved using, among others, neutralization, antibody profiles or IIFT biochip mosaics (e.g., Euroimmun Arbovirus Fever Mosaics and Arbovirus Profiles; Fig. 3.2; Table 3.6).

The **Euroimmun Anti-Chikungunya Virus IIFT** with CHIKV-infected and uninfected cell substrates coated on separate biochips is a valuable tool for the diagnosis of CHIKV infections and antibody seroprevalence studies. Evaluation of this assay for the detection of IgM antibodies revealed a sensitivity and specificity of 96.9% and 98.3%, respectively. The IgG IIFT showed a sensitivity of 95.4% and a specificity of 100% [53]. Another study demonstrated increasing IgM assay sensitivity along with the progression of the disease, improving from 75.6% by day five to 100% by day seven, at a specificity of 100% [98]. Assay accuracy for anti-CHIKV IgM detection amounted to 96–97%, which was similar to the accuracy demonstrated for commercial IgM ELISAs (95–100%) [43]. Other Euroimmun IIFT biochip mosaics are suitable for differential diagnosis analyses, such as Arbovirus Fever Mosaics and Arbovirus Profiles (Fig. 3.2, Tables 3.2, 3.6 and 3.7).

The **Euroimmun Anti-Chikungunya Virus ELISA** is based on a recombinant CHIKV-specific structural protein. When compared to other commercial anti-CHIKV assays, it was shown to provide excellent overall agreement as well as very high sensitivity and specificity of up to 100% depending on the cohort (Fig. 3.3) [43, 68].

## 3.4 Summary

Biochip mosaics for IIF-based detection of antibodies against ZIKV, DENV and CHIKV enable the determination of the infectious agent by means of titration. Alternatively, the combination of the highly specific and non-cross-reactive NS1-based anti-ZIKV ELISA with anti-DENV- and CHIKV-ELISAs allows for reliable differential diagnosis, also in regions where these infections are co-endemic.

**Table 3.6** Available serological tests for the diagnosis of CHIKV infections (11/2016)

| Company | Assay | Ig class | Format | References |
|---|---|---|---|---|
| Abcam | Human Anti-Chikungunya Virus IgM ELISA | IgM | ELISA | [43] |
| | Human Anti-Chikungunya Virus IgG ELISA | IgG | ELISA | |
| ADI | Human Anti-Chikungunya virus (CHIKV) IgM capture ELISA | IgM | ELISA | |
| | Human Anti-Chikungunya virus (CHIKV) IgG capture ELISA | IgG | ELISA | |
| | Human Anti-Chikungunya virus E1 (CHIKV-E1) IgM capture ELISA | IgM | ELISA | |
| | Human Anti-Chikungunya virus E1 (CHIKV-E1) IgG capture ELISA | IgG | ELISA | |
| | Human Anti-Chikungunya virus E2 (CHIKV-E2) IgM capture ELISA | IgM | ELISA | |
| | Human Anti-Chikungunya virus E2 (CHIKV-E2) IgG capture ELISA | IgG | ELISA | |
| Artron | Chikungunya IgG/IgM Antibody | IgM/IgG | ICA (RDT) | |
| Biocan | Chikungunya IgG/IgM Antibody | IgM/IgG | ICA (RDT) | |
| Biocan | Zika/Dengue/Chikungunya Combo Tests | IgM/IgG | ICA (RDT) | |
| CD | Chikungunya IgM ELISA | IgM | ELISA | |
| | Human Chikungunya IgG ELISA | IgG | ELISA | |
| | Chikungunya Virus IgG capture ELISA | IgG | ELISA | |
| CTK | RecombiLISA CHIK IgM Test | IgM | ELISA | [43] |
| | OnSite Chikungunya IgM Combo Rapid Test | IgM | ICA (RDT) | [11, 43, 68] |
| | OnSite Duo Dengue IgG/IgM-CHIK IgM Rapid Test | IgM | ICA (RDT) | |
| DA/CD | AccuDiag Chikungunya IgM ELISA | IgM | ELISA | |
| | AccuDiag Chikungunya IgG ELISA | IgG | ELISA | |
| Diasorin | Chikungunya Virus IgM μ-capture | IgM | ELISA | |
| DRG | Chikungunya IgM | IgM | ELISA | |
| | Chikungunya IgG | IgG | ELISA | |
| Euroimmun | Anti-Chikungunya virus IIFT (IgG or IgM) | IgM/IgG | IIFT | [43, 53, 58, 97, 98] |
| | Arbovirus Fever Mosaic 1 (IgG or IgM) | IgM/IgG | IIFT | |
| | Arbovirus Fever Mosaic 2 (IgG or IgM) | IgM/IgG | IIFT | |
| | Arbovirus Profile 3 (IgG or IgM) | IgM/IgG | IIFT | |
| | Anti-Chikungunya Virus ELISA (IgM) | IgM | ELISA | [43, 68] |
| | Anti-Chikungunya Virus ELISA (IgG) | IgG | ELISA | [68] |
| GenWay | Chikungunya IgM μ-capture ELISA | IgM | ELISA | [43] |
| | Chikungunya IgG capture ELISA | IgG | ELISA | |
| IBL | Chikungunya IgM μ-capture ELISA | IgM | ELISA | [68] |
| | Chikungunya IgG capture ELISA | IgG | ELISA | [68] |
| InBios | CHIKjj Detect IgM ELISA | IgM | ELISA | [43] |
| | CHIKjj Detect IgG ELISA | IgG | ELISA | |
| J. Mitra & Co | Advantage Chikungunya IgM Card | IgM | ICA (RDT) | |
| LumiQuick | QuickProfile Chikungunya IgG/IgM Combo Test | IgM/IgG | ICA (RDT) | |
| Mikrogen | alphaWell Chikungunya IgM μ-capture | IgM | ELISA | |
| | alphaWell Chikungunya IgG | IgM | ELISA | |
| | recomLine Tropical Fever IgM | IgM | LIB | |
| | recomLine Tropical Fever IgG | IgG | LIB | |
| MyBioSource | Chikungunya Virus IgM μ-capture ELISA | IgM | ELISA | |
| | Chikungunya Virus IgG capture ELISA | IgG | ELISA | |
| NovaTec | NovaLisa Chikungunya Virus IgM μ-capture ELISA | IgM | ELISA | [49] |
| | NovaLisa Chikungunya Virus IgG capture ELISA | IgG | ELISA | [49] |

(continued)

**Table 3.6** (continued)

| Company | Assay | Ig class | Format | References |
|---|---|---|---|---|
| OrangeLife | OL Chikungunya IgM | IgM | ICA (RDT) | |
| R-Biopharm | RIDASCREEN Chikungunya Virus IgM μ-capture | IgM | ELISA | |
| | RIDASCREEN Chikungunya Virus IgG capture | IgG | ELISA | |
| SD Standard/ Alere | Chikungunya IgM ELISA | IgM | ELISA | [9, 43] |
| | BIOLINE Chikungunya IgM | IgM | ICA (RDT) | [9, 43, 44, 68] |
| Vircell | ZIKV-DENV-CHIKV IFA IgM | IgM | IIFT | |
| | ZIKV-DENV-CHIKV IFA IgG | IgG | IIFT | |

*ELISA* enzyme-linked immunosorbent assay, *ICA* immunochromatographic assay, *IIFT* indirect immunofluorescence test, *LIB* line immunoblot, *RDT* rapid diagnostic test

**Table 3.7** Suppliers of serological assays (supplement to Tables 3.1, 3.5 and 3.6)

| Notation | Company |
|---|---|
| Abcam | Abcam plc, Cambridge, UK |
| Access Bio | Access Bio, Inc., Somerset, NJ, USA |
| ADI | Alpha Diagnostic International., Inc., San Antonio, TX, USA |
| Artron | Artron Laboratories, Inc., Burnaby, BC, Canaca |
| Biocan | Biocan Diagnostics, Inc, Coquitlam, BC, Canada |
| Bio-Rad | Bio-Rad Laboratories, Marnes La Coquette, France |
| Biosynex | Biosynex, Strasbourg, France |
| Calbiotech | Calbiotech, Inc., El Cajon, CA, USA |
| Chembio | Chembio Diagnostic Systems, Inc., Medford, NY, USA |
| CD | Creative Diagnostics, Shirley, NY, USA |
| CDC | CDC, Centers for Disease Control and Prevention, Atlanta, GA, USA |
| Core | Core Diagnostics, Birmingham, UK |
| CTK Biotech | CTK Biotech Inc., San Diego, CA, USA |
| DA/CD | Diagnostic Automation/Cortez Diagnostics, Inc., Woodland Hills, CA, USA |
| DIACHECK | Dr. Julio Moran, Laboratories, Herrliberg (Zurich), Switzerland |
| Dia.Pro | Dia.Pro, Diagnostic Bioprobes Srl., Sesto San Giovanni (MI), Italy |
| DiaSorin | DiaSorin, Saluggia, Italy |
| DRG | DRG Diagnostics, Marburg, Germany |
| Euroimmun | Euroimmun, Lübeck, Germany |
| Focus | Focus Diagnostics, Cypress, CA, USA |
| GenWay | GenWay Biotech Inc., San Diego, CA, USA |
| IBL | IBL International, Hamburg, Germany |
| InBios | InBios International Inc., Seattle, WA, USA |
| J. Mitra & Co | J. Mitra & Co. Pvt. Ltd., New Delhi, India |
| LumiQuick | LumiQuick Diagnostics, Inc., Santa Clara, CA, USA |
| MIKROGEN | MIKROGEN, Neuried, Germany |
| MP | MP Biomedicals, Santa Ana, CA, USA |
| MyBioSource | MyBioSource, Inc., San Diego, CA, USA |
| NovaTec | NovaTec Immundiagnostica, Dietzenbach, Germany |
| Omega | Omega Diagnostics Ltd., Alva, UK |
| OrangeLife | OrangeLife, Rio de Janeiro, Brazil |
| Orgenics/Alere | Orgenics Ltd. (Alere Inc.), Yavne, Israel |
| Panbio/Alere | Panbio Diagnostics (Alere Inc.), Brisbane, Australia |
| PROGEN | PROGEN Biotechnik, Heidelberg, Germany |
| R-Biopharm | R-Biopharm AG, Darmstadt, Germany |
| SD Standard/ Alere | SD Standard Diagnostics, Inc. (Alere Inc.), Yongin-si, Gyeonggi-do, Republic of Korea |
| SD Biosensor | SD Biosensor, Inc., Suwon-si, Gyeonggi-do, Republic of Korea |
| Viramed | Viramed Biotech AG, Planegg, Germany |
| Vircell | Vircell, Granada, Spain |
| Viro-Immun | Viro-Immun Diagnostics, Oberursel, Germany |
| Virion\Serion | Virion\Serion, Würzburg, Germany |
| Zephyr | Zephyr Biomedicals, Verna, Goa, India |

(continued)

## Discussion of Chapter 3 in *Dengue and Zika: Control and Antiviral Treatment Strategies*

*This discussion was held at the 2nd Advanced Study Week on Emerging Viral Diseases at Praia do Tofo, Mozambique.*
*Transcribed by Hilgenfeld R and Vasudevan SG (Eds); approved by Dr. Claudia Ohst.*

**Aruna Sampath:** Most of the tests that you have shown are based on serology. Do you have any tests that can specifically look at infectious virus particles?

**Claudia Ohst:** The only test that we have is the Dengue NS1 test, that shows the NS1 for all four types when the virus breaks down. We only have this test for Dengue virus and not for Zika virus or Chikungunya virus. We do not do any PCRs either.

**Aruna Sampath:** Ok, so for the Dengue, what sensitivity do you have? What is the sensitivity for the NS1 antigens?

**Claudia Ohst:** Our Dengue NS1 test looks into detecting the Dengue NS1 antigen, so it is not an antibody ELISA here, but an antigen test. Compared to the antibody ELISA, the correlation is quite good and even slightly more sensitive. This has been confirmed by serology where both, the antibody and the NS1 antigen test have been performed.

**Subhash Vasudevan:** And do you think you can find the serotype as well?

**Claudia Ohst:** No. This one is for all of the four, so you cannot distinguish the serotypes with this, it is just a screening test.

**Kerstin Falk:** But you have this mosaic slide with Dengue 1, 2, 3, and 4. But then you said you have to titrate it out. And how much work is that and how reliable is that?

**Claudia Ohst:** You see, in immunofluorescence you always have crossreactions between the different serotypes and all of them will come up positive in your screening dilution. That's why you have to titrate. Obviously you can take bigger steps and just see where you end up. And then the one with the highest titer should be the virus in your serotype that you are dealing with. Maybe PCR is the better method, but at least there is an option to do this via serology.

**Kerstin Falk:** So what antigen are you using? So what part of the genome? For the Dengue 1, 2, 3 and 4?

**Claudia Ohst:** So we are using Dengue 2, I think. For the NS1 antigen ELISA we used antibodies from all four Dengue serotypes, for the antibody ELISA we used purified Dengue 2 virus particles (now purified particles from all four Dengue types).

**Kerstin Falk:** But when you have Dengue 1, 2, 3 and 4?

**Claudia Ohst:** Yes, but it does detect all of them.

**Aravinda de Silva:** But you have done this with IFA. IFA is immunofluorescence.

**Claudia Ohst:** Well, the IFA uses infected cells, there are four Biochips on each field of the Mosaic slide, each chip is coated with cells which were infected with only one of the four Dengue serotypes.

**Aravinda de Silva:** The Zika virus data looks very encouraging, much better than Dengue.

**Claudia Ohst:** We put a lot of work into it.

Aravinda de Silva: Have you looked at Zika virus in late convalescence, in other words, in people 6 months, 1 year after exposure and see how the NS1 antibodies change over the long term?

**Claudia Ohst:** I think, these data might just be on their way. You need to get the specimens for this. And everything is from the first half basically of 2016 and that is when we start to get the specimens. I cannot give an answer now.

**Aravinda de Silva:** The other question I have had is that you showed that in secondary cases the Zika IgM, you could not really detect, it was not very sensitive. Is it because there is no IgM or is it because the IgG antibodies are binding to your NS1 antigen, because you have very high IgG levels. And are the IgG antibodies binding the NS1 and preventing IgM from binding?

**Claudia Ohst:** No. There apparently is not very much IgM. What we do in our IgM tests, is to pre-absorb all the IgG, so they should not

really interfere with the assay. We really only catch the IgM antibodies that are present in your specimen.

**Aravinda de Silva:** So even in the commercial assay, you pre-absorb the IgG?

**Claudia Ohst:** Yes. So our dilution solution provided in the test kit contains something that pre-absorbs the IgGs.

**George Gao:** I missed your NS1 antigen preparation. Did you use the mammalian-expressed protein or insect cells expressed proteins for your antigen, NS1 antigen? How do you prepare the antigen?

**Claudia Ohst:** Are you referring to the NS1 antigen for Dengue or for Zika?

**George Gao:** For both. Dengue 1–4 and the Zika. How do you prepare the antigen?

**Claudia Ohst:** For Dengue, it is an antigen capture test, so obviously we coat our ELISA plates with antibodies that catch the antigen. In the case of Zika, where we detect antibodies against the NS1 antigen, Zika NS1, we have NS1 protein coated on our plate. And this protein is prepared by our company, in our own molecular biology department that produces these recombinant antigens.

**Paul Young:** I am a little confused by the IgM and IgG antibody data against Zika. NS1. In Dengue, in primary infected patients at least, there is very little if any, and In many cases no, early IgG or IgM response to NS1. This comes along much later in convalescence. Very strong anti-E and sometimes prM, but very little NS1. Is it completely different in Zika? The patients actually mount a good antibody response to the NS1 protein?

**Claudia Ohst:** Well, we are using the NS1, because that is the one where we really get specific results. Otherwise it would not really help.

**Paul Young:** I understand completely, but it is not there in Dengue, so I am just surprised.

**Claudia Ohst:** I cannot tell you. You see, in our Dengue ELISAs, we do not even use the Dengue NS1. That might be the reason why.

**Paul Young:** Yes, that is why no one uses it. And I am just very surprised that Zika infection would actually induce such a high response, given that the viral load is lower in Zika virus. I suspect, no one has really done that analysis yet. And I know there is really quantitative data on the amount of NS1 in patients, but I suspect it is lower than in Dengue virus. So again it is still confusing.

**Claudia Ohst:** Maybe we were just lucky with our NS1.

**Jonas Schmidt-Chanasit:** I would support this comment, because I think it was neglected that the sensitivity especially for IgG is not that good. In combination with IgM, this Zika assay is okay, but if you only perform the IgG, you lose like 30–40% sometimes. Then, what is also important to mention is that the plaque titers do not correlate with the reactivity in the Zika virus ELISA. So you have sometimes very high viral titers and the ELISA is completely negative. So it is quite interesting and it might be related to what was just mentioned before. I just wanted to highlight that. High sensitivity is only achieved if you do this in combination, IgG and IgM together. We have a lot of samples and this was also very nicely illustrated by the data from Mozambique, that they were completely negative in IgG ELISA, but positive in the IFA. So this is due to a lack of sensitivity.

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