Improved and customized dengue serodiagnostics through combined NS1/IgM testing and novel dual-cut-off IgG ELISA

euroimmun
From Revvity

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1 Introduction

Accurate diagnostics of dengue virus (DENV) infection are essential for patient management, outbreak control, and vaccine implementation. Serological testing plays a key role, especially when molecular assays are unavailable or viremia subsides; yet, cross-reactivity with other flaviviruses remains a challenge. This study examined the diagnostic accuracy of four Euroimmun ELISAs, including a newly developed dual-cut-off Anti-DENV IgG ELISA.

2 Methods

The Dengue Virus NS1 ELISA, Anti-Dengue Virus Type 1-4 ELISA (IgM), Anti-Dengue Virus Type 1-4 ELISA (IgG; native antigen/gE-based), and the novel Anti-Dengue Virus NS1 ELISA 2.0 (IgG; recombinant NS1-based, with an alternative higher cut-off for use in flavivirus-endemic regions) were analyzed. Sensitivity was determined using sera from 22 Vietnamese patients with RT-PCR-confirmed DENV infection, collected during acute (t1, 1–6 dpo), early convalescent (t2, 4–9 dpo), and late convalescent (t3, 13–19 dpo) phases. Specificity was assessed with samples from 500 healthy German blood donors (HBD) and 40 patients each with West Nile virus (WNV) or Zika virus (ZIKV) infection, all serologically positive for the respective virus.

3 Results

Sensitivities determined with the four ELISAs were 90.5%/70.0%/0% (t1/t2/t3) for NS1, 33.3%/85.0%/77.3% for IgM, 66.7%/100%/100% for IgG, 33.3%/65.0%/100% for IgG 2.0 using the standard cut-off, and 19.1%/50.0%/100% for IgG 2.0 using the alternative cut-off. Combined NS1 and IgM testing achieved 100% sensitivity in single acute-phase samples (Table 1, Figure 1). Overall ELISA specificity amounted to 85.7% (HBD/WNV/ZIKV: 95.0%/50.0%/5.0%) for IgG, compared to 95.7% (98.2%/95.0%/65.0%) for IgG 2.0 using the standard cut-off and 99.5% (99.8%/97.5%/97.5%) for IgG 2.0 using the alternative cut-off, as summarized in Table 2 and Figure 2.

Table 1: Sensitivities determined in samples from 22 patients with PCR-confirmed DENV infection; numbers in brackets indicate positive/borderline/negative results; borderline results were considered negative for sensitivity calculations; [a] three samples (t1: n = 1; t2: n = 2) were excluded because they were not available in sufficient quantity for serological testing in this study; [b] combined positivity was defined as positive reactivity in at least one of the two ELISAs; [c] an alternative cut-off (20 RU/mL) may be applied for samples from flavivirus-endemic areas instead of the standard cut-off (10 RU/mL); dpo, days post onset; NA, not applicable; ND, not determined at this time

Sampling time: range	n	DENV RT-PCR (reference)	DENV NS1 ELISA	Anti-DENV Type 1-4 ELISA (IgM)	Combined NS1+IgM [b]	Anti-DENV Type 1-4 ELISA (IgG)	Anti-DENV NS1 ELISA 2.0 (IgG) [c]	
							Standard cut-off	Alternative cut-off
t1: 1–6 dpo	21 [a]	NA (21/0/0)	90.5% (19/0/2)	33.3% (7/2/12)	100%	66.7% (14/1/6)	33.3% (7/0/14)	19.1% (4/1/16)
t2: 4–9 dpo	20 [a]	ND	70.0% (14/0/6)	85.0% (17/2/1)	85.0%	100% (20/0/0)	65.0% (13/1/6)	50.0% (10/1/9)
t3: 13–19 dpo	22	ND	0% (0/0/22)	77.3% (17/1/4)	77.3%	100% (22/0/0)	100% (22/0/0)	100% (22/0/0)

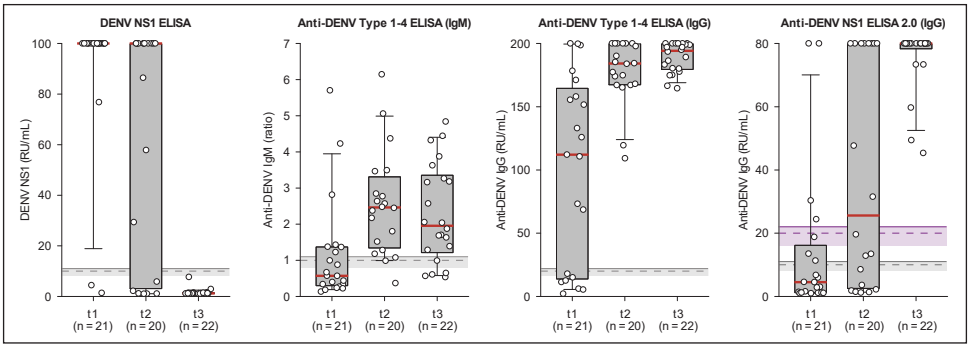

Figure 1: Distribution of DENV NS1, anti-DENV IgM and anti-DENV IgG levels; boxes indicate interquartile ranges (outer bounds) and medians (bold red lines); whiskers present the 90th and 10th percentiles; the dashed line shows the assay-specific cut-off, the adjacent shaded area indicates the borderline range, and the solid horizontal line marking the upper limit of the borderline range represents the positivity threshold; the alternative cut-off of the Anti-DENV NS1 ELISA 2.0 (IgG) is indicated in purple

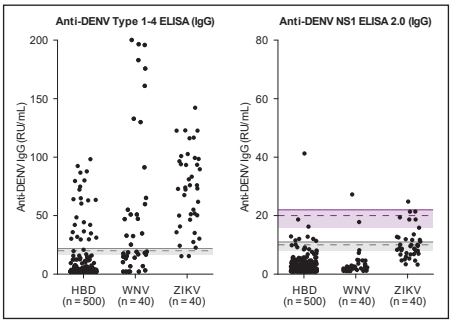

Figure 2: Reactivity of Anti-DENV IgG ELISAs in samples from healthy blood donors (HBD) and patients infected with West Nile virus (WNV) or Zika virus (ZIKV), all serologically positive for the respective virus; refer to the legend of Figure 1 for explanation of line styles and graphical conventions

Table 2: Specificity of Anti-DENV IgG ELISAs; numbers in brackets indicate positive/borderline/negative results; borderline results were considered negative for specificity calculations; HBD, healthy blood donors; WNV, West Nile virus; ZIKV, Zika virus

Panel	n	Anti-DENV Type 1-4 ELISA (IgG)	Anti-DENV NS1 ELISA 2.0 (IgG)	
			Standard cut-off	Alternative cut-off
HBD	500	95.0% (25/3/472)	98.2% (9/10/481)	99.8% (1/2/497)
WNV	40	50.0% (20/7/13)	95.0% (2/1/37)	97.5% (1/1/38)
ZIKV	40	5.0% (38/0/2)	65.0% (14/12/14)	97.5% (1/5/34)
Total	580	85.7% (83/10/487)	95.7% (25/23/532)	99.5% (3/8/569)

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4 Conclusion

Euroimmun ELISAs support customized, highly accurate and versatile diagnostic strategies applicable to various dengue testing contexts. Combining NS1 and IgM ELISAs offers an alternative to molecular assays during the acute phase of infection. The native antigen/gE-based IgG ELISA enables early sensitive IgG detection, although with limited specificity. With minimal cross-reactivity, the NS1-based dual-cut-off ELISA 2.0 (IgG) reliably captures DENV-specific IgG dynamics and enhances differentiation from other flaviviruses, which could provide an advantage in the use for convalescent-phase diagnostics, surveillance, and pre-vaccination serostatus determination.

Outcome

- **DENV NS1 ELISA + Anti-DENV Type 1-4 ELISA (IgM): 100% sensitivity in acute phase**
- **Anti-DENV Type 1-4 ELISA (IgG): highly sensitive in early-convalescent phase**
- **Anti-DENV NS1 ELISA 2.0 (IgG): highly sensitive in late-convalescent phase, highly specific**

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