# Validation of the NovaLisa Kit to detect IgM antibodies of Zika virus in different samples

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## 1 Background

Zika virus (ZIKV),+ss RNA virus, is an arthropod borne flavivirus primarily transmitted by *Aedes* mosquitoes. ZIKV was discovered in a rhesus monkey (Uganda) in 1947. It was an ignored virus, but ZIKV emerged suddenly on large scale in Micronesia (2007) and Polynesia (2013). ZIKV rapidly caused an epidemic in many South and Central American and Caribbean countries in 2015 .
It has been reported that ZIKV occurred in Dengue virus exposed areas, that is why the diagnosis of ZIKV is being complicated due to the chance of cross reactivity. In current study, we have tried to validate an ELISA to evaluate its sensitivity and specificity against ZIKV IgM antibody.

## 2 Sample

We selected serum samples from patients with dengue IgM (n= 3), IgG dengue (n= 7), yellow fever vaccination (n=10), malaria positive (n=2), CMV IgG (n=4), negative samples (n=13), acute ZIKV infection urine (n=15) and sera (n=15), pregnancy cohort (n=20) from the hospital (AZP)

## 2 ELISA Procedure

- Diluted serum samples (1/100 in sample diluent) were added to 96 well plate coated with anti-human IgM along with both controls and substrate blank.
- Plates were incubated 1 hour ± 5 minutes at 37◦C in the dark after covering the well with the provided foil.
- After the incubation step, wells were aspirated and washed three times with washing buffer
- After adding conjugate, plates were incubated for 30 minutes at 37◦C in the dark
- Plates were washed 3X with washing buffer and Tetramethylbenzidine (TMB substrate solution) was dispensed to all wells.
- Plates were incubated at RT for 15 min (Dark) and reaction was stopped with $H_2SO_4$.
- Finally absorbance was measured at wavelength 420/ 620 as reference filter.

## 3 Results

| Cohort | samples | Zika negative samples | Zika IgM positive samples |
|---|---|---|---|
| Dengue IgM positive patients | 3 | 3 | 0 |
| Dengue IgG positive patients | 7 | 5 | 2 |
| Yellow fever vaccination | 10 | 9 | 1 |
| CMV IgG positive patients | 4 | 4 | 0 |
| Malaria positive patients | 2 | 2 | 0 |
| Pregnancy cohort | 10 | 9 | 1 |
| Zikavirus PCR positive sera | 15 | 12 | 3 |
| Zikavirus PCR positive urine | 15 | 10 | 5 |
| Negative patients | 13 | 13 | 0 |

With the NovaLisa kit we found that n=12 of the collected samples were ZIKV IgM positive. Among ZIKV IgM positive samples, n=2 were denque IgG positive, n=8 were ZIKV RT-PCR positive samples (n=5 serum and n=3 urine), n=1 pregnancy cohort, n=1 yellow fever vaccinated

## 4 Conclusion

During the evaluation of the Novalisa kit we did not find any cross reactivity with other flaviviruses or parasites. Detection of ZIKV IgM in two dengue IgG positive and yellow fever samples may be due to the secondary exposure of ZIKV. The ZIKV IgM positive pregnant woman had ZIKV symptoms before blood sampling. Although the kit is usually used for human serum or plasma, but in current study we found ZIKV IgM antibodies in urine as well, which suggests the broad spectrum detection ability of the selected kit.

## 5 References

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