# *Anti-Zika virus IgA may indicate an acute infection in anti-Zika virus IgM-negative patients.*

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# Do we really need other test parameter for ZIKV?

**We have…**

- ≤ d5 Direct pathogen detection via ZIKV-specific rt RT-PCR in serum/ plasma -> extended time window for urine

- \> d5 anti-ZIKV IgM detection (NS1 or full virus) + FU neutralisation testing

- Significant IgG titer increase or seroconversion indicating acute infection

- For later stages ( e.g. pregnancy, sexual transmission) anti-ZIKV IgG

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**Assays are limited by…**

- rt RT-PCR: low viremia with short time window sometimes at LoD of assays -> risk of false negative results; *Corman et al., 2016*

- Anti-ZIKV IgM can be low, delayed or absent in secondary Flavivirus infections; *Steinhagen et al., Poster presentation* in the exhibition area

- Full virus based anti-ZIKV IgM detect pan-Flavi antibodies; *Zika MAC ELISA EUA*

- Serum of patients with previous Flavivirus infections contain cross neutralizing antibodies e.g. DENV to YFV up to 80%; *Houghton-Trivino et al., 2008*
 -> During secondary infections cross-reactive AB may significantly contribute to neutralization of the current infecting virus; *Puschnik et al., 2013*

# Case report

ID: Z1042972430; country of infection: Colombia
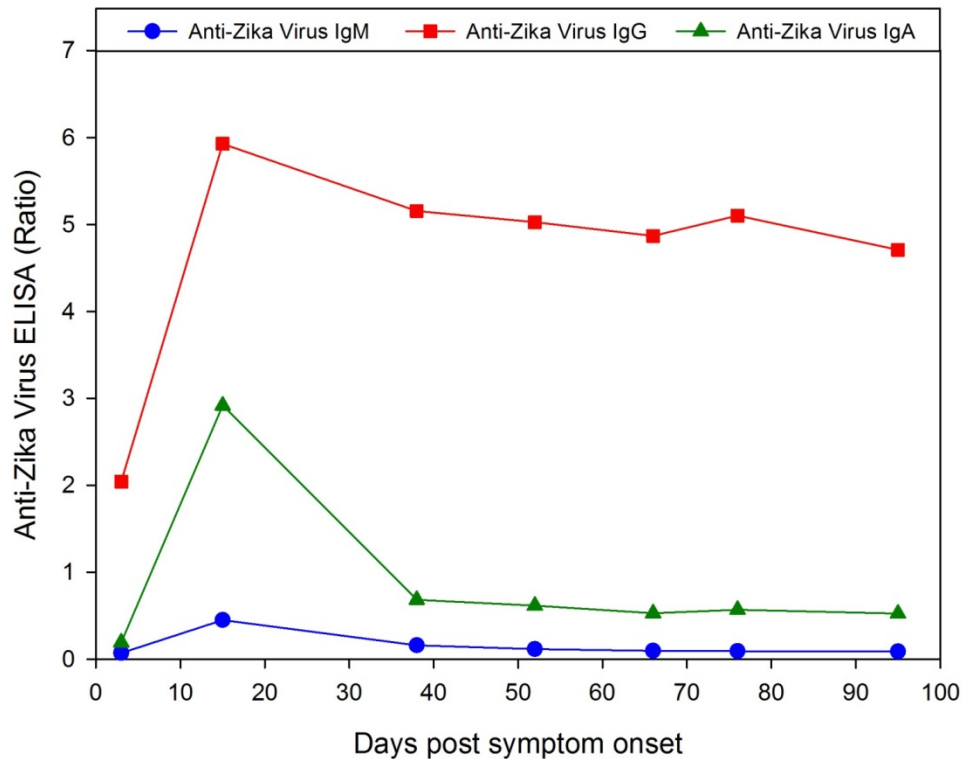


**Is Anti-Zika virus IgA capable of indicating an acute infection in anti-Zika virus IgM-negative patients?**

# Anti-Zika virus IgA may indicate an acute infection in anti-Zika virus IgM-negative patients

K. Steinhagen[1], N. Wilhelm[1], J. Schmidt-Chanasit[2,3], P. Emmerich[2], D. Huzly[4], E. Lattwein[1], J. Fraune[1], W. Schlumberger[1], and J. Warnecke[1]

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[3]German Center for Infection Research (DZIF), Partner Site Hamburg-Luebeck-Borstel, Hamburg, Germany
[4]Institute for Virology, Medical Center – University of Freiburg, Germany
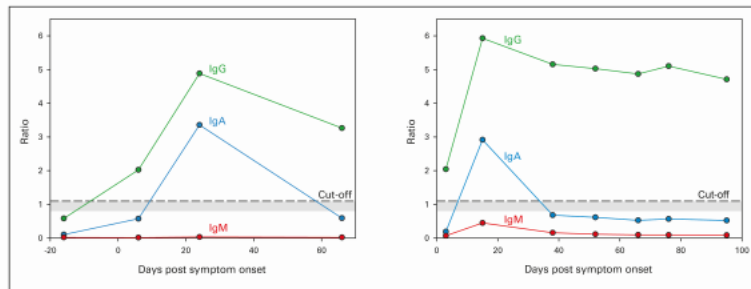
| Patient | Country of origin | Country of infection | Days after symptoms | Anti-ZIKV IgA ratio; pos: ≥1.1 | Anti-ZIKV IgM ratio; pos: ≥1.1 | Anti-ZIKV IgG ratio; pos: ≥1.1 |
|---|---|---|---|---|---|---|
| 1 | Colombia | Colombia | -16 | 0.1 | 0.0 | 0.6 |
| | | | 6 | 0.6 | 0.0 | 2.0 |
| | | | 24 | 3.4 | 0.0 | 4.9 |
| | | | 66 | 0.6 | 0.0 | 3.3 |
| 2 | Colombia | Colombia | 3 | 0.2 | 0.1 | 2.0 |
| | | | 15 | 2.9 | 0.5 | 5.9 |
| | | | 38 | 0.7 | 0.2 | 5.2 |
| | | | 52 | 0.6 | 0.1 | 5.0 |
| | | | 66 | 0.5 | 0.1 | 4.9 |
| | | | 76 | 0.6 | 0.1 | 5.1 |
| | | | 95 | 0.5 | 0.1 | 4.7 |
| 3 | Germany | Martinique | 11 | 4.1 | 1.0 | 0.1 |
| | | | 16 | 9.0 | 2.7 | 1.4 |
| | | | 36 | 1.5 | 1.2 | 2.5 |
| 4 | Germany | Nicaragua | 4 | 0.3 | 0.2 | 0.2 |
| | | | 9 | 7.6 | 2.4 | 1.0 |
| | | | 30 | 2.4 | 0.9 | 3.0 |

## Introduction

In secondary flavivirus infections, specific IgM is often found at low or undetectable titers, while synthesis of specific IgG is rapidly stimulated. Shortly after infection the IgG titer levels off being indistinguishable from IgG titers in convalescent infections. The same has been observed in patients with **Zika virus (ZIKV)** infections from regions endemic for other flaviviruses. Since ZIKV infection is suspected to be associated with major neurological consequences, differentiation between acute (secondary) and past infections is crucial. We analysed the course of anti-ZIKV IgA, IgM and IgG titers in sequential serum samples of four patients, investigating whether IgA might indicate an acute phase of infection.

## Methods

Serum samples were taken at several time points from two Columbians with a presumptive background of past flavivirus infections and from two German travellers, all presenting with confirmed ZIKV infections.

Titers of anti-ZIKV IgM and IgG were measured using a commercial NS1-based Anti-Zika virus ELISA (cut-off ≥ 1.1, Euroimmun AG, Germany). An indirect immunofluorescence test (Arbovirus Fever Mosaic 2, IgM, cut-off ≥ 1:10, Euroimmun AG, Germany) based on cells infected with ZIKV was used additionally for IgM measurement. For determination of anti-ZIKV IgA, the ELISA was adapted, applying an anti-human IgA conjugated with peroxidase. The cut-off was set to a ratio of 1.1.

## Results

In the German travellers, anti-ZIKV IgM was detected with ELISA as well as IIFT at day 9 and day 16, respectively. Active infections were confirmed by subsequent anti-ZIKV IgG seroconversion. Anti-ZIKV IgA measurements were above 1.1 in all samples except for one, showing an initial increase and a subsequent decrease.

In the sequential samples of the Colombian patients, measurements of IgM antibodies against ZIKV-NS1 antigen in ELISA were persistently below the cut-off. In accordance, testing for IgM against full ZIKV in IIFT was negative in all but one, weak positive sample (1:10). Anti-ZIKV IgG was positive already within the first week in both patients. IgA, however, showed a titer increase, peaking above the cut-off in week three and four before dropping below the threshold again.

## Conclusion

When specific IgM is not detectable neither with NS1- nor full virus-based assays as observed in the Colombian patients, measurement of anti-ZIKV IgA may allow discrimination of acute from past infections.

# Primary vs secondary Flavivirus infections
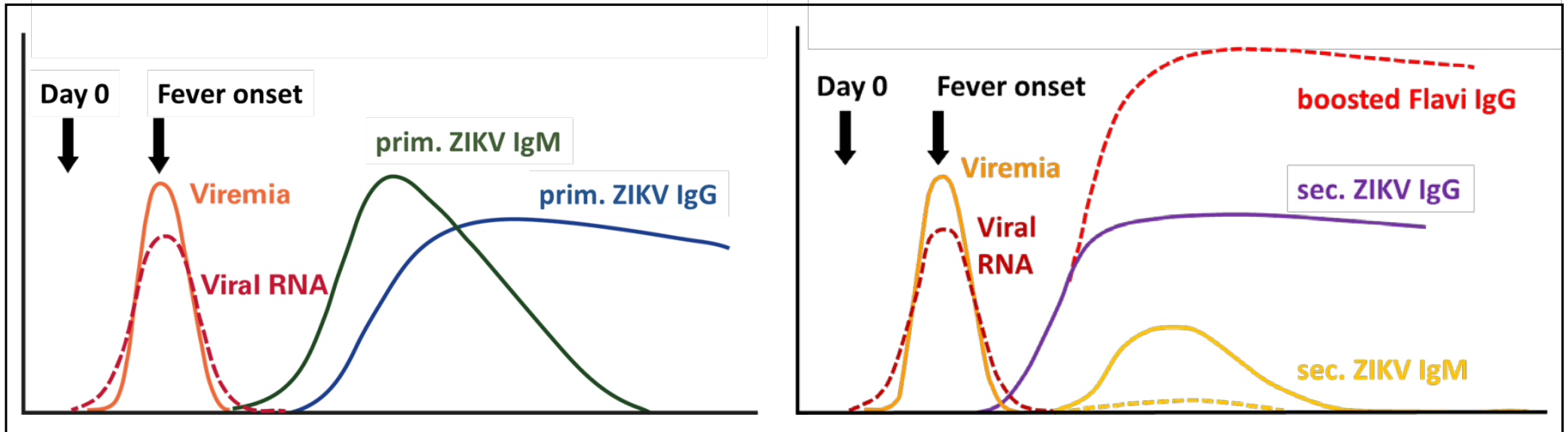


## Study requirements

- rt RT-PCR to secure infecting virus (direct pathogen detection)

- Follow up samples for NS1-based anti-ZIKV <u>IgG and IgM</u> (specific anti-ZIKV)

- Full virus based anti-ZIKV IgG and IgM IFT (presence of cross reacting AB)

- Neuralization test (IgG confirmatory)

- <u>Research parameter:</u> NS1-based anti ZIKV IgA ELISA

# Study: Basic parameter

- **n=31**
- **Residents of Dominican Republic**
- **Collection date Apr. – July 2016**
- **Clinical signs of Arbovirus infection (fever, rash …)**
- **Average 35 y**
- **84% female**
- **10 pregnancies**
- **First bleeding ≤ d5 post onset of symptoms (p.o.s.)**
- **Follow up bleeding 5-10 days later**
- **rt RT-PCR confirmed acute ZIKV infection**

# Confirmation of ZIKV infection ≤ d5 p.o.s.

| n = 31 (1st bleed) | | MolBiol ZIKV-specific rt RT-PCR |
|---|---|---|
| | | positive |
| RealStar® Zika Virus RT-PCR Kit ALTONA Diagnostics | positive | 25 |
| | borderline | 0 |
| | negative | 6 |

**Test system: anti-Zika Virus ELISA**
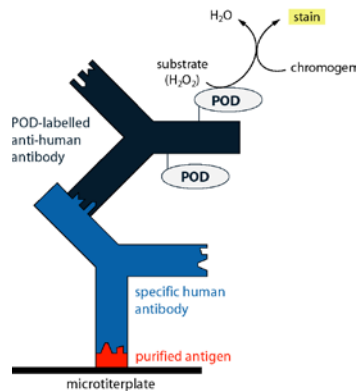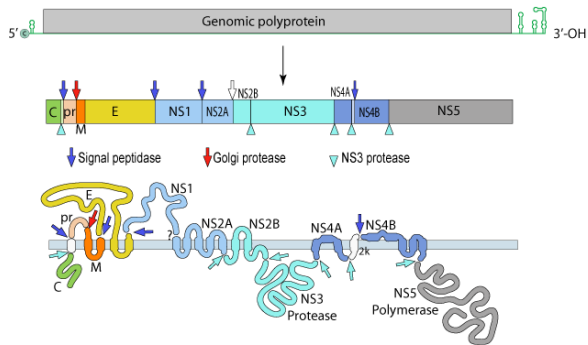


ZIKV NS1-antigen for high specificity



Indirect ELISA for high throughput and robustness

ELISAs for **IgG, IgM  and IgA** to allow seperate determination of both antibody classes

# Anti-ZIKV specific antibodies > d5 p.o.s.

| n = 31 | | MolBiol ZIKV-specific rt RT-PCR |
|---|---|---|
| | | positive |
| EUROIMMUN Anti-Zika Virus ELISA IgM | positive | 9 |
| | borderline | 6 |
| | negative | 16 |

**15/ 31 patients (48%) detected**
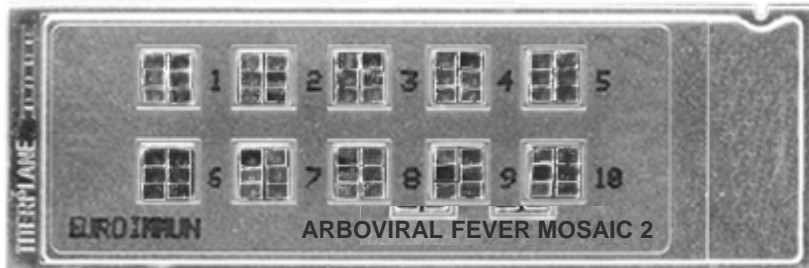**-> No ZIKV-specific IgM antibodies?**

**Test system IFA: Arbovirus Fever Mosaic 2**

Combination of cell cultures infected with different arboviruses in one incubation field using BIOCHIP technology -> **full virus based** IFA



FI 2668-####-1 G/M

# Anti-Flavivirus antibodies > d5 p.o.s.

**Test system IFA: Arbovirus Fever Mosaic 2**

Combination of cell cultures infected with different arboviruses in one incubation field using BIOCHIP technology -> **full virus based** IFA
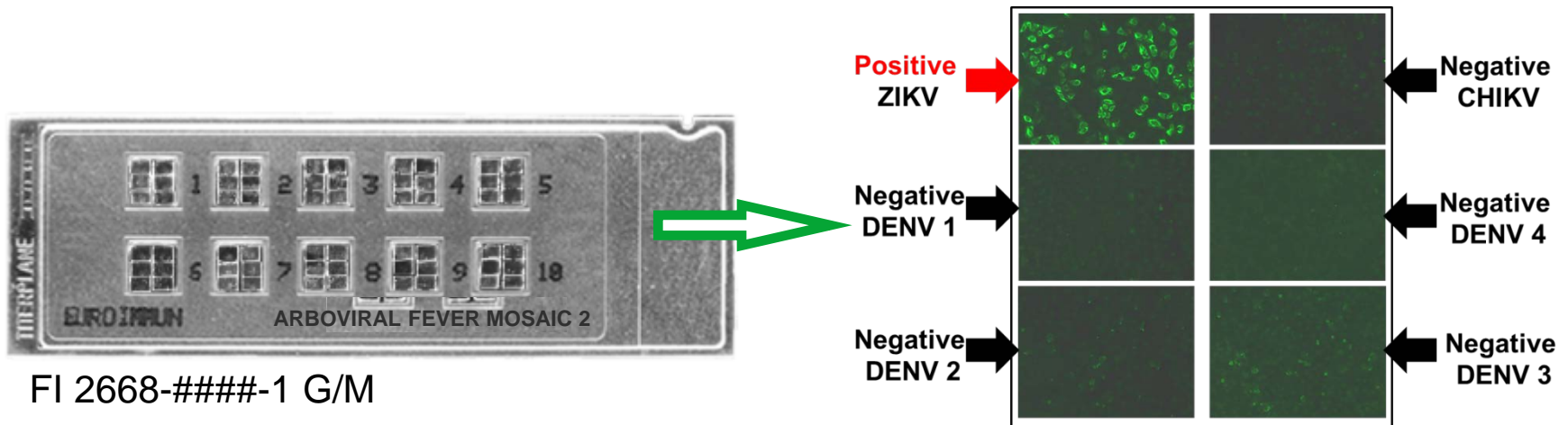


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IFA **endpoint titrations** are used to discriminate between specific and cross-reacting antibodies: **Highest titer = causative virus**

# Anti-Flavivirus antibodies > d5 p.o.s.

| n = 31 | | MolBiol ZIKV-specific rt RT-PCR |
|---|---|---|
| | | positive |
| **EUROIMMUN Arboviral Fever Mosaic 2** | positive | 30 |
| | borderline | 0 |
| | negative | 1 |

**Full-virus based detection is more sensitive but…**

**13/ 31 (42%) sera contain polyspecific anti-Flavivirus**

**antibodies type IgM that react with DENV <u>and</u> ZIKV virus**

**-> infecting virus <u>undetermined!</u>**

| n = 31 | | MolBiol ZIKV-specific rt RT-PCR |
|---|---|---|
| | | positive |
| ZIKV neutralization test | positive | 31 |
| | borderline | 0 |
| | negative | 0 |

**Neutralization test finds 100% of patients …**

**-> after 1 week lab labour in BSL 2 …**

**-> accute infection or previous/ convalescent?**

**-> specificity**

# Anti-Flavivirus antibodies sequential bleedings

| n = 31 | | MolBiol ZIKV-specific rt RT-PCR |
|---|---|---|
| | | positive |
| **EUROIMMUN Anti-Zika Virus ELISA IgG** | **≥2-fold titer increase** | 17 |
| | **seroconversion** | 14 |
| | **negative** | 0 |

Testing both samples for
a)  two-fold IgG titer increase or
b)  IgG seroconversion

**-> 100% detection rate**
**-> detects rt RT-PCR negative samples**
**-> some samples needed dilution measurements**

# Anti-ZIKV specific antibodies > d5 p.o.s.

| n = 31 | | MolBiol ZIKV-specific rt RT-PCR positive |
|---|---|---|
| **Anti-Zika Virus ELISA IgA** | positive | 29 |
| | borderline | 0 |
| | negative | 2 |

**Anti-ZIKV IgA detection by NS1-based ELISA detects 29/31 (94%) of patients.**

# Anti-ZIKV specific antibodies > d5 p.o.s.

| n = 31 | | MolBiol ZIKV-specific rt RT-PCR<br><br>positive |
|---|---|---|
| **Anti-Zika Virus ELISA IgA** | positive | 29 |
| | borderline | 0 |
| | negative | 2 |

**Anti-ZIKV IgA detection by NS1-based ELISA detects 29/31 (94%) of patients.**

**Anti-Zika Virus IgA negative sera were positive for IgM, amounting to 100% for a combined IgA and IgM testing**

## Open questions and limitations

❑ **ZIKV rt RT-PCR positive is pre-selection bias**

❑ **Primary Flavivirus infections?**

❑ **Kinetics of anti-ZIKV IgA development**

❑ **Specificity in case of acute other Flavivirus infections**

# Acknowledgements

Institute for Experimental Immunology, EUROIMMUN AG, Germany:
**Katja Steinhagen, Nadja Wilhelm, Erik Lattwein, Johanna Fraune**
**Wolfgang Schlumberger**

WHO Collaborating Center for Arbovirus and Haemorrhagic Fever Reference and Research, Bernhard-Nocht Institute for Tropical Medicine, Hamburg and
German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel, Germany:
Germany:
**Jonas Schmidt-Chanasit, Petra Emmerich**

Institute for Virology, Medical Center – University of Freiburg, Germany:
**Daniela Huzly**

Thank you for your attention