EVALUATION OF EUROIMMUN ZIKA VIRUS IGG AND IGM ELISA KITS

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BACKGROUND

- Zika has become a global phenomenon in the last 8 years
- Association with congenital abnormalities has secured its promenient place on a world stage
- "Vector borne rubella"
- Laboratory diagnosis is critical to clinical management of cases particularly in pregnancy or in those wanting to become pregnant
- Laboratory diagnosis of flaviviruses comes with layers of difficulty

DIAGNOSIS

- Virus isolation
- PCR
- Serology
  - HI
  - IFA
  - ELISA

  Cross reactive
  - CF — More specific
  - Neutralisation — Most specific

OBJECTIVES

- Examine the sensitivity and specificity of Euroimmun kits
- Determine kit suitability for routine diagnostic use in Asia/Pacific region.
- Provide interpretative guidelines for reporting these results

METHODS

- Two part evaluation
  - Retrospective
  - Prospective
- Retrospective
  - Samples referred specifically for Zika testing (or other flavivirus testing where Zika was a possibility) were used to determine sensitivity, specificity, positive and negative predictive values and likelihood ratios
  - Specificity was checked using samples positive for other flaviviruses including: dengue (primary & secondary infections); Japanese encephalitis virus; Yellow Fever virus; Murray Valley encephalitis virus, Kunjin, Kokobera, Stratford, Alfuy, Edge Hill viruses

RETROSPECTIVE EVALUATION

- IgM specificity was further challenged with samples from patients with EBV, CMV and Q fever infections and positive ANA titres (these latter results were not used for the calculation of diagnostic test statistics).
- 310 samples were tested for IgG (100 positive for Zika) and 320 samples were tested for IgM (100 positive for Zika).
- All samples were tested by the Euroimmun IgG ELISA, Euroimmun IgM ELISA, in house IgG indirect fluorescent antibody (IFA), in house IgM IFA and neutralisation using a 90% endpoint.
- All neutralisation positive samples were retested for IgM and IgG by neutralisation following removal of IgG or IgM, respectively. The neutralisation result was used as the reference standard.

RESULTS

IgG Test

	Confirmed Positive	Confirmed Negative
Euroimmun positive	91	25
Euroimmun negative	9	185
Total	100	210

Sensitivity 91% Specificity 88.1%
NPV 95.4% PPV 78.5%
Negative LHR 0.10 Positive LHR 7.64

IgM test

	Confirmed Positive	Confirmed Negative
Euroimmun positive	89	17
Euroimmun negative	11	203
Total	100	220

Sensitivity 89.0% Specificity 92.3%
NPV 95.0% PPV 84.0%
Negative LHR 0.12 Positive LHR 11.52

PROSPECTIVE EVALUATION

- No attempt is made to choose samples
- Patient's are tested because clinicians request them
- On-going but already false positives have been found in both IgG and IgM
- Continuing for another 2 to 3 months

CONCLUSIONS

- Euroimmun assays did not provide the very high levels of specificity that were reported in initial evaluations by others.
- Cross reactions were more common with IgG than IgM but were found in both antibody classes.
- Cross reactions with dengue were the most common and cross absorption studies indicate that cross reaction between dengue and Zika goes in both directions.
    - This is concerning in the Asia Pacific region where multiple flaviviruses co-circulate, and for countries which have active vaccination programmes for Japanese encephalitis virus or Yellow fever virus.
- The prevalence of Zika virus seropositivity in this study was relatively high, at 32%, as our laboratory receives samples for primary testing, for confirmatory testing from other laboratories and international referrals from endemic areas.
- In a routine diagnostic laboratory in Australia, the expected seroprevalence may be much lower, which would have an impact on the expected positive predictive value. For example, if the samples tested had a lower seroprevalence of 10%, the positive predictive values of the Euroimmun ELISA assays for IgM and IgG would be 56% and 46% respectively (though this would also be affected by the background seroprevalence of cross-reacting flaviviruses in the test population).

CONCLUSIONS

- Early infections were missed by the Euroimmun assays in some cases.
- Compared with IFA IgG, Euroimmun IgG was slightly delayed appearing as late as 21 days post onset.
- Euroimmun kits are potentially useful as a first line test to screen out negatives particularly if two suitably timed samples are used.
- Positive IgM and/or IgG should be submitted for confirmatory testing against a panel of flaviviruses.
- False positive fourfold rises in Zika antibody titres due to cross reactions were also noted in both IgG and IgM.

CONCLUSIONS

- Sequential bleeds suggests that IgM can be detected for approximately 6 to 8 weeks if the infection is a primary flavivirus infection but in secondary infection IgM was not always detected and when present was detectable for shorter periods and at lower level, as is seen in other flavivirus infections.
- IgG positive only samples and it is suggested that comments include the following or similar: "These results may suggest past infection with Zika virus. Cross reaction with another flavivirus or flavivirus vaccination cannot be excluded. If further differentiation is required neutralisation testing may be necessary".
- False positive IgMs were noted with non-arboviral conditions (i.e. CMV, EBV and ANA) – this is more common in indirect ELISA formats than is seen in antibody class capture IgM assays using monoclonal antibodies.

CLINICAL CASE

- 27 yr female returned from Singapore, 10 wks pregnant, was 2 wks pregnant while in Singapore, no clinical illness worried about Zika
- Past history of dengue as a child
- IgG IFA 40, IgG Euroimmune pos; IgM neg by both IFA & Euroimmune
- Neut titre 10

CLINICAL CASE

- 37 yr female returned from Carribean in April. 7mths preg now – worried about Zika as scans show foetus has small head.
- IgG IFA 640 IgG Euroimmune pos; IgM neg by both IFA & Euroimmune
- Neut titre 2560

CLINICAL CASE

- 40yr female pregnant following IVF
- Was in Brazil early in pregnancy – told she had Zika wanted confirmation prior to termination
- IgG IFA 40 IgG Euroimmune pos; IgM neg by IFA & equivocal by Euroimmune
- Neut titre <10