Zika Virus Antibody Detection: Evaluation of Three Different Serologic Methodologies

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Abstract

Background: The Zika virus (ZV) epidemic in South America was declared an international public health emergency by the World Health Organization in early 2016 due to association with fetal microcephaly and post-infectious neurologic manifestations. Among acutely symptomatic individuals, fever, arthralgia, conjunctivitis and maculopapular rash are common, and overlap with those of other arbovirus infections, including dengue (DV) and Chikungunya (CHIKV). Notably, ZV, DV, and CHIKV co-infections are possible due to the shared Aedes species mosquito vector and co-endemicity in South America. Serologic testing for ZV is currently available only through public health departments and the CDC.

We evaluated the Euroimmun (Lübeck, Germany) ZV EIA and IFA and the Viramed (Planegg, Germany) line immunoassay (LIA) for detection of anti-ZV IgM and IgG antibodies.

Methods: The Euroimmun and Viramed assays were evaluated using 96 specimens from the following groups: a) symptomatic Colombian (serum n=47; plasma n=5) or Brazilian (plasma; n=3) patients, b) asymptomatic pregnant women (n=17) with recent travel to a ZV endemic area and c) 24 specimens submitted for DV or CHIKV testing to Mayo Medical Laboratories. 25 specimens were tested by the CDC ZV MAC-ELISA. Reference range studies were performed using 50 normal donor sera from Minnesota residents (including 20 sera submitted for routine prenatal care) and specificity was evaluated using three panels: a) 32 sera positive for IgM antibodies to West Nile virus (WNV; n=10), DV (n=16), CHIKV (n=4) or St. Louis Encephalitis virus (SLEV; n=2), b) 44 sera positive for IgG antibodies to WNV (n=13), DV (n=20), CHIKV (n=5) or SLEV (n=6), and c) 20 sera from patients with confirmed DV infection.

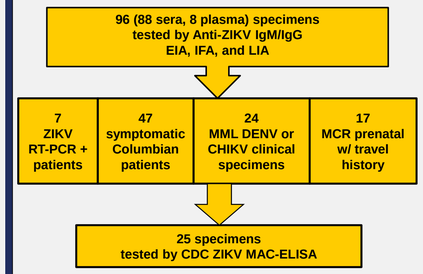
Results: The LIAs were considered the comparator method due to absence of a routinely available gold standard assay for detection of anti-ZV IgM. The ZV IgM EIA and IFA had a positive, negative and overall agreement of 66.7% (2/3), 93.5% (87/93), 92.7% (89/96) and 66.7% (2/3), 95.7% (88/92), 94.7% (90/95), respectively, versus the ZV IgM LIA. The ZV IgG EIA and IFA showed a positive, negative and overall agreement of 93.9% (31/33), 98.4% (61/62), 96.8% (92/95) and 100% (33/33), 82.3% (51/62), 88.4% (84/95), respectively, compared to the ZV IgG LIA. 25 sera (8 ZV IgM EIA positive and 17 prenatal ZV IgM EIA negative) were tested by the CDC ZV MAC-ELISA, and showed 100% and 76.0% concordance with the ZV IgM EIA and LIA, respectively.

All assays had 100% specificity among normal donors except the IgG EIA (98%; 49/50), IFA (94%; 47/50) and LIA (98%; 49/50). The ZV IgM EIA, IFA, and LIA showed 96.9% (31/32), 90.6% (29/32), and 100% (32/32) specificity, respectively, in sera from panel A. The ZV IgG EIA, IFA, and LIA showed 86.4% (38/44), 52.3% (23/44) and 84.1% (37/44) specificity, respectively in sera from panel B. The majority (76.5%) of cross-reactivity occurred in DV IgG positive samples. The ZV IgG EIA was negative in all 20 sera collected from patients residing in a non-ZV endemic region who were DV RT-PCR and IgG positive.

Conclusions: The Euroimmun and Viramed ZV IgM assays showed high specificity for ZV in this evaluation. The ZV IgM EIA showed excellent overall agreement with the CDC MAC-ELISA, suggesting that this assay may be used as an alternative screening test for ZV; positive specimens should continue to be confirmed at the CDC. The ZV IgG EIA reactivity observed in samples positive for DV IgG may be due to ZV/DV co-infection or a false positive DV or ZV IgG result. While further studies to evaluate cross-reactivity of both IgM and IgG ZV assays are needed, ZV IgG EIA reactivity was not observed in DV confirmed patients from a non-ZV endemic area.

Study Methods

Accuracy Study Specimens:



Abbreviations:
ZIKV, Zika virus; DENV, dengue virus; CHIKV, Chikungunya virus; MML, Mayo Medical Laboratories ; MCR, Mayo Clinic Rochester

Serologic Assays

Euroimmun AG (Lübeck, Germany):

- Anti-ZIKV (IgM/IgG) Enzyme Immunoassay (EIA)
- Arboviral Fever Mosaic 2 (IgM/IgG) Immunofluorescence assay (IFA)
  - Mosaic includes: ZIKV, DENV (serotypes 1-4), and CHIKV infected cells

Viramed Biotech AG (Planegg, Germany)

- ZIKV ViraStripe® (IgM/IgG) Line Immunoassay (LIA)

CDC MAC-ELISA

- Performed at the Minnesota Department of Health - Public Health Laboratory (St. Paul, MN) and CDC - Division of Vector-Borne Infectious Diseases (Fort Collins, CO)

All assays were performed per manufacturer's instructions for use.

Results

Table 1. Comparison of the Euroimmun EIA and Viramed LIA for detection of anti-ZIKV IgM Ab

N = 96		LIA IgM Pos.	LIA IgM Neg.	LIA IgM Ind.	Positive Agreement	Negative Agreement	Overall Agreement
EIA IgM	Pos.	2	1	5	66.7%	93.5%	92.7%
	Neg.	1	83	3			
	Ind.	0	0	1			

Table 2. Comparison of the Euroimmun EIA and CDC MAC-ELISA for detection of anti-ZIKV IgM Ab

N = 25		CDC MAC-ELISA Pos.	CDC MAC-ELISA Neg.	Positive Agreement	Negative Agreement	Overall Agreement
EIA IgM	Pos.	8[a]	0	100.0%	100.0%	100.0%
	Neg.	0	17[b]			

[a] Clinical history available for 6/8 patients (see Table 3)
[b] 17 specimens from asymptomatic Mayo Clinic (Rochester, MN) pregnant women with recent travel to a Zika endemic area

Table 3. Clinical History of 6 Patients that tested positive for Anti-ZIKV IgM Ab by the Euroimmun EIA and CDC MAC-ELISA

Pt.	Sex	Age	Zika Virus Signs and Symptoms: Fever	Skin Rash	Joint Pain	Myalgia	HA	Conjunctivitis
#1	F	37	+	+	+	+		+
#2	M	46	+	+		+		
#3	F	32	+	+				
#4	F	N/A		+		+	+	
#5	M	15	+	+		+	+	+
#6	F	24	+	+	+	+	+	+

Table 4. Comparison of the Euroimmun EIA and Viramed LIA for detection of anti-ZIKV IgG Ab

N = 95		LIA IgG Pos.	LIA IgG Neg.	LIA IgG Ind.	Positive Agreement	Negative Agreement	Overall Agreement
EIA IgG	Pos.	31	1	0	93.9%	98.4%	96.8%
	Neg.	1	60	0			
	Ind.	1	1	0			

Table 5. Comparison of the Euroimmun IFA and Viramed LIA for detection of anti-ZIKV IgM and IgG Ab

N = 95		LIA IgM Pos.	LIA IgM Neg.	LIA IgM Ind.	Positive Agreement	Negative Agreement	Overall Agreement
IFA IgM	Pos.	31	1	0	93.9%	98.4%	96.8%
	Neg.	1	60	0			
	Ind.	1	1	0			
		LIA IgG					
IFA IgG	Pos.	33	11	0	100.0%	82.3%	88.4%
	Neg.	0	51	0			
	Ind.	0	0	0			

Table 6. Summary of Results for Potentially Cross-Reacting Specimens

Analyte Present	No. Tested	% Nonreactive Specimens: EIA IgM	IFA IgM	LIA IgM
Anti-WNV IgM	10	100	100	100
Anti-DENV IgM	16	93.8	81.3	100
Anti-CHIKV IgM	4	100	100	100
Anti-SLV IgM	2	100	100	100
Healthy prenatal	20	100	100	100
Healthy donor	30	100	100	100
Total	**82**	**98.8**	**96.3**	**100**
		EIA IgG	**IFA IgG**	**LIA IgG**
Anti-WNV IgG	13	100	76.9	100
Anti-DENV IgG	20	70.0	30	70
Anti-CHIKV IgG	5	100	40	80
Anti-SLEV IgG	6	100	83.3	100
Healthy prenatal	20	100	95	100
Healthy donor	30	96.7	93.3	96.7
Total	**94**	**92.6**	**74.5**	**91.5**

Table 7. EIA Reactivity in Individuals from a Zika Non-Endemic Region with Early Convalescent Stage Dengue Infection

Specimen Category	No. Tested	*Number of Positive Specimens by Method (%)*: DENV-PCR/NS1	DENV-IgM/IgG	ZIKV-IgG	ZIKV-IgM
Dengue w/ Warning Signs[a]	7	7	7	0	0

[a] 2009 WHO Classification - dengue w/ warning signs; specimens collected > 5 days post onset of fever (Singapore; June and September 2012)

Table 8. EIA Reactivity in Individuals from a Zika Non-Endemic Region with Suspected Secondary Dengue Infection

Specimen Category	No. Tested	*Number of Positive Specimens by Method (%)*: DENV-PCR/NS1	DENV-IgG	ZIKV-IgG	ZIKV-IgM
Secondary Dengue Infection[a]	13	13	13	0	0

[a] 2009 WHO Classification - dengue w/ warning signs; specimens collected ≤ 5 days post onset of fever (Singapore; May 2010 to September 2012)

Conclusions

- The anti-ZIKV IgM Euroimmun EIA and Viramed LIA showed high analytical specificity for ZIKV during this evaluation.
- The anti-ZIKV IgM EIA demonstrated excellent overall agreement (100%; n=25) with the CDC MAC-ELISA.
- Anti-ZIKV IgG EIA reactivity was not observed in DENV confirmed-positive patients from a Zika virus non-endemic region.
- Anti-ZIKV IgG EIA reactivity observed in specimens positive for anti-DENV IgG may be due to ZIKV/DENV co-infection or false positive ZIKV or DENV IgG results. Further studies focusing on the specificity of these assays are warranted.

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