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Performance evaluation of the Diasorin LIAISON® XL Zika capture IgM CLIA test

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ABSTRACT

Because Zika virus (ZIKV) can cause serious birth defects and is involved in cases of Guillain-Barré syndrome, the ZIKV outbreak in the American continent in 2015 resulted in an enormous need for ZIKV diagnostic tools. We evaluated the LIAISON® XL Zika Capture IgM test on 106 samples from patients, mainly travelers, with a confirmed or probable ZIKV infection. Sensitivity between 0 and 84 days after onset of symptoms was 92.5%. Specificity was evaluated on a panel of 56 samples known to cause possible cross-reactions. Cross-reaction with DENV antibodies was limited (10.5%) but false-positive results occurred in samples from patients with malaria, CMV and EBV infections.

1. Introduction

Zika virus (ZIKV) is a mosquito-borne flavivirus closely related to other flaviviruses such as dengue virus (DENV), yellow fever virus (YFV), tick-borne encephalitis virus (TBEV) and Japanese encephalitis virus (JEV). It is mainly transmitted by mosquitos of the genus *Aedes*. Additional ways of transmission are via blood transfusion, perinatal, congenital and sexual. In April of 2015 a huge ZIKV outbreak started in the American continent, spreading from Brazil to other parts of Southern and Central America, the Caribbean and Florida and Texas in the US (World Health Organization n.d.-a). So far, no vector borne autochthonous ZIKV infections have been observed in Europe, sexual transmission has however been described (Spiteri et al., 2015). Clinical symptoms were reported in only 20% of people in the outbreak on Yap island (Duffy et al. 2009) but recent studies suggested that asymptomatic ZIKV infections occur in only 27–30% of travelers (de Laval et al. 2016; Diaz-Menendez et al. 2018).

Because of the involvement of ZIKV in cases of Guillain-Barré and more importantly in a congenital syndrome (World Health Organization n.d.-b) the ZIKV outbreak in the American continent resulted in an enormous need for diagnostic tools to detect ZIKV infections. Consequently, various new diagnostic tests have been launched onto the market. Many reverse-transcriptase polymerase chain reaction (RT-PCR) assays, based on different ZIKV targets, have been described (Charrel et al. 2016). The incubation period of ZIKV infections ranges from 3 to 12 days (Loos et al. 2014). The period of virus detection is short with viral RNA detectable in serum only in the first week after onset of symptoms and in urine till 14–21 days post-symptom onset (pso) (Bingham et al. 2016; https://ecdc.europa.eu/en/zika-virus-infection/surveillance-and-disease-data/case-definition 2016). In pregnant women, the virus may persist longer (Driggers et al. 2016).

After the acute phase, diagnosis relies on serology. Immunoglobulin M antibodies appear 4 days pso and remain detectable for 2–12 weeks, whereas IgG antibodies appear shortly after and persist for months to years (Landry 2017).

Several commercial assays have been launched to detect ZIKV antibodies. Recently a new chemiluminescent immunoassay (CLIA) has been introduced onto the market.

The aim of this study was to investigate the performance of the new LIAISON® XL Zika Capture IgM test (Diasorin S.p.A., Saluggia, Italy) on well-characterized samples from ZIKV-infected patients and to challenge the test against a panel of samples known to cause possible cross-reactions.

2. Materials and methods

2.1. Samples and laboratory diagnosis

A total of 162 left-over serum samples were selected for this retrospective evaluation. One hundred and forty-two samples, collected in

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the Institute of Tropical Medicine (ITM) (Antwerp, Belgium) between August 2015 and December 2017 were either from patients that presented at the outpatient clinic of the ITM or were submitted by Belgian laboratories to the clinical laboratory of Clinical Biology of ITM, in the scope of its function as the national reference center for arboviruses. The remaining 20 samples were from the microbiology laboratory at the University Hospital of Brussels.

The first panel consisted of 106 samples from 89 patients. Ninety-two samples from 77 patients with a confirmed and 5 samples from patients with a probable ZIKV infection were classified based on the European Centre of Disease Prevention and Control laboratory criteria and, in the absence of IgM antibodies, the remaining 9 samples from 7 patients were classified as confirmed based on Viral Neutralization Test (VNT) in combination with the ECDC clinical and epidemiological criteria (a recent stay in a ZIKV endemic area) (https://ecdc.europa.eu/en/zika-virus-infection/surveillance-and-disease-data/case-definition 2016).

Laboratory confirmation was done by ZIKV RNA detection by PCR in a clinical specimen (11 samples, 9 patients), detection of ZIKV specific IgM antibodies and confirmation by a VNT (66 samples, 57 patients), both PCR/VNT (24 samples, 18 patients) or seroconversion or four-fold increase in the titer of ZIKV specific antibodies in paired serum samples (1 sample). The laboratory criterion for a probable case was detection of ZIKV specific IgM antibodies in serum.

The majority of samples were collected from females (60%). As far as known, none of them was pregnant. Selection of samples was mainly based on onset of symptoms which was between 0 and 84 days before sample collection (n = 94). The remaining 12 (10 confirmed and 2 probable) infections were asymptomatic (n = 2) or the date of symptom onset was not known (n = 10). The vast majority of samples (97/106, 91.5%) was obtained from travelers to countries where ZIKV is endemic, 5 (4.7%) were from residents/patients visiting friends and relatives (VFR) and 5 (4.7%) samples derived from patients with unknown travel history.

Laboratory diagnosis was performed as previously described (Van den Bossche et al. 2019). Molecular detection was performed until 5 days pso. Detection of ZIKV IgG and IgM antibodies was performed by the Euroimmun ZIKV ELISA (Euroimmun, Lübeck, Germany) according to the manufacturer's instructions on samples drawn more than 5 days pso, samples from asymptomatic patients and patients for whom no clinical information was available. In case of presence of ZIKV IgG antibodies and a negative result for ZIKV IgM antibodies and symptom onset or exposure less than 2 months before, detection of ZIKV IgM antibodies was additionally performed by an ImmunoFluorescence Assay (IFA, Arbovirus Fever Mosaic 2, Euroimmun, Lübeck, Germany). On all samples with positive or indeterminate results for ZIKV IgG, a ZIKV VNT was performed. In case of presence of IgM antibodies in the absence of IgG antibodies, a follow-up sample was requested for VNT.

A second panel consisted of 26 serum samples expecting to cause possible cross-reactions, due to the presence of high levels of rheumatoid factor (RF) (range: 50–298 IU/mL, n = 3), a *Plasmodium sp.* infection based on positive thick smear and PCR (n = 8) or serology (n = 1), presence of IgM antibodies against cytomegalovirus (CMV) (n = 2) or Epstein–Barr virus (EBV) (n = 3) or presence of anti-DENV IgM (n = 3, two of which were also positive with DENV PCR) or both IgM and IgG antibodies (n = 6) antibodies.

Based on the results of panel 2, a third panel of 30 samples was added to further evaluate possible cross-reactivity. This panel consisted of 10 samples from patients with anti-DENV IgM (n = 2) or both IgM and IgG (n = 8) antibodies collected at the ITM and 10 samples each from patients with a confirmed CMV or EBV infection collected in the microbiology laboratory at the University Hospital of Brussels containing both IgM and IgG antibodies except one sample containing anti-CMV IgM but no IgG antibodies. No travel history to ZIKV endemic areas was known for these patients. Seven samples out of the 10 with a confirmed CMV infection derived from pregnant women.

2.2. LIAISON® XL Zika Capture IgM test

The LIAISON® XL Zika Capture IgM test is a fully automated commercially available in vitro NS1-based diagnostic CLIA, designed for the detection of anti-ZIKV IgM antibodies. Anti-ZIKV IgG antibodies (ZIKV-C) are measured and used for the interpretation of the ZIKV-M results but not reported. The IgM-result is reported negative when the ZIKV-M index is <1.0 with any value for the ZIKV-C index or when ZIKV-M is ≥1.0 to <2.2 with a ZIKV-C index <4.0. Zika IgM is presumed positive when ZIKV-M yields an index ≥1.0 to <2.2 with a ZIKV-C index ≥4.0 or ZIKV-M index ≥2.2 with any possible value for the ZIKV-C index. Indices are calculated based on the obtained Relative Light Unit (RLU) values, compared to the calibration curve RLU values. The analyses were performed on the LIAISON® XL analyzer in the University Hospital of Brussels.

2.3. Statistical analysis

A Wilcoxon rank sum test was performed using the R software® (version 3.4.3; Vienna, Austria). Sensitivities with 95% confidence intervals were calculated with the Analyze-it® for Excel software (version 2.2.6; Leeds, UK).

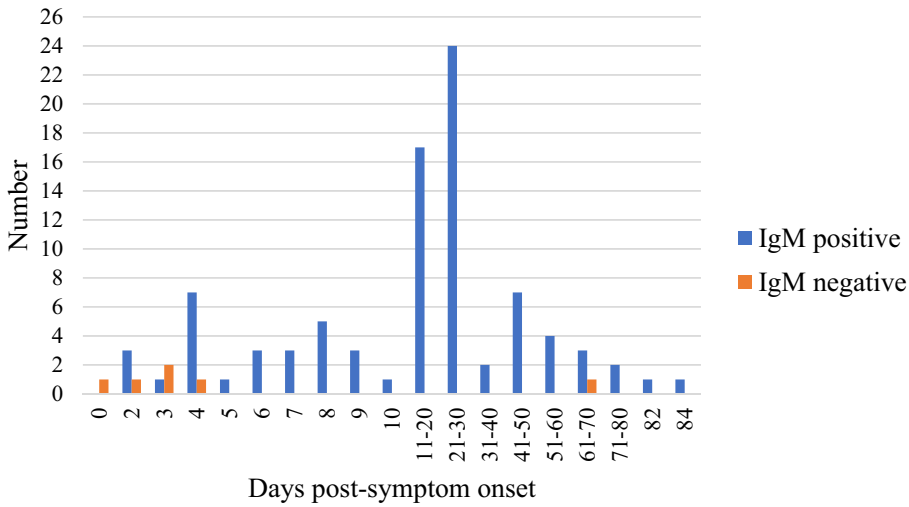

Fig. 1. Positive and negative results of the LIAISON® XL Zika Capture IgM test in relation to the number of days post-symptom onset (n = 94).

Table 1
Sensitivity of the LIAISON® XL Zika Capture IgM test to detect IgM antibodies per time period in samples from patients with a confirmed ZIKV infection (n = 94).

Days post-symptom onset	n	Range	Median	#positive LIAISON® XL Zika Capture IgM	Sensitivity (95% CI)
≤5	17	0–5	4	12/17	0.71 [0.44–0.90]
6–29	52	6–28	17	52/52	1.00 [0.93–1.00]
≥30	25	30–84	50	24/25	0.96 [0.80–1.00]

2.4. Ethics

The study was approved by the Institutional Review Board of the ITM (reference number 1232/18).

3. Results

Out of the 101 samples from patients with a confirmed ZIKV infection, 94 (93.1%) were positive with the LIAISON® XL Zika Capture IgM test. Six out of 7 samples with a negative result were from 0 to 4 (n = 5) and 63 (n = 1) days pso (one unknown) confirmed by PCR or VNT respectively. In addition, 4 out of 5 samples (80.0%) from patients with a probable ZIKV infection were positive. The negative result was scored in a sample with IgM antibodies only. This resulted in a sensitivity of the LIAISON® XL Zika Capture IgM of 92.5% (98/106). For comparison, considering borderline results as positive, the sensitivity of the Euroimmun Anti-Zika IgM virus ELISA in the same group of samples was 66.0% (70/106).

Results of 94 samples collected from patients with a confirmed (n = 91) or probable (n = 3) ZIKV infection for which the date of onset of symptoms is known are shown in Fig. 1. Sensitivity between 5 and 84 days pso, was 76/77 (98.7%). Sensitivity of the LIAISON® XL Zika Capture IgM test per time period are shown in Table 1.

The results of panel 2 are shown in Table 2. The LIAISON® XL Zika Capture IgM test was positive in 2/9 samples with DENV antibodies, both containing only DENV IgM and no IgG antibodies, 1/3 EBV and 2/2 CMV IgM antibody positive samples and 7/9 samples from patients with malaria. Additional VNT and IFA could exclude a true ZIKV infection in the two cross-reacting CMV positive samples and in 1 of the 2 *Plasmodium* infected samples with a Liaison ZIKV-M index of >29. In the other samples, additional ZIKV PCR, IgM IFA and VNT could not reveal a true ZIKV infection.

Table 3 shows the results of panel 3. None of the 10 samples obtained from patients with a DENV infection resulted in a positive result for ZIKV IgM on LIAISON® XL. On the other hand, the test revealed a positive result in 5/10 (50.0%) and 6/10 (60.0%) of samples from patients with an EBV or CMV infection respectively. No additional tests could be performed to confirm or exclude a true ZIKV infection.

The ratios of ZIKV-M in the group of cross-reacting samples (n = 23) were significantly lower than the ratios in samples from patients with confirmed and probable ZIKV infections (n = 98) ($p < 0.01$).

4. Discussion

As the viremic phase during which molecular testing for ZIKV is useful, is short (except for pregnant women that may have a prolonged viremia) and the viremic phase may go unnoticed, diagnosis of Zika often relies on serological tests.

The evaluation of the LIAISON® XL Zika Capture IgM CLIA on a panel of 106 samples from patients with certain and probable ZIKV infections from 0 to 84 days pso revealed a sensitivity of 92.5%. This is higher than the 74% sensitivity reported by Sloan et al. in a population of Canadian travelers to ZIKV endemic areas conducted on samples from between 2 and 161 days pso (Sloan et al. 2018). A possible explanation might be that the selection of samples for our study was based on our laboratory strategy making use of the Euroimmun IgM and IgG ELISA tests, with a reported suboptimal sensitivity when used as screening tests (Basile et al. 2018; Lustig et al. 2017).

Reported sensitivities of the NS1-based Euroimmun Anti-Zika virus ELISA were 37–53% in comparable diagnostic windows (Basile et al. 2018; Safronetz et al. 2017). Sensitivities up till 68% were reported in panels limiting the number of days pso (Lustig et al. 2017; Steinhagen et al. 2016). The low sensitivity and short duration of positivity of the Euroimmun IgM ELISA is the reason why the IFA Arbovirus Fever Mosaic 2 was added to our diagnostic portfolio already soon after the start of the ZIKV outbreak and why IgM and IgG detection should be combined (Van den Bossche et al. 2019). Sensitivity of the NS1 based NovaLisa® Zika IgM μ-capture ELISA (NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) was 65% between 2 and 118 and 69.8% between 1 and 164 days pso (Basile et al. 2018; Safronetz et al. 2017). Reported sensitivities of E-protein based tests such as the CDC-based MAC ELISA (100%) and the Zika Virus Detect MAC-ELISA (InBios International Inc., Seattle,

Table 2
Assessment of cross-reactivity (n = 26) due to DENV (n = 9), EBV (n = 3), CMV (n = 2), rheumatoid factor (RF, n = 3), *P. falciparum* (n = 5), *P. vivax* (n = 2), *P. ovale* (n = 1), *P. malariae* (n = 1).

Possible cross-reaction	n	LIAISON® XL Zika Capture IgM		
		POSITIVE (n)	ZIKV-M	ZIKV-C
DENV	9	2	3.86	0.340
			3.78	0.394
EBV IgM	3	1	4.64	0.500
CMV IgM	2	2	7.96	0.315
			3.28	0.351
RF (>50 IU/mL)	3	0	N/A	N/A
P. falciparum	5	4	20.3	0.491
			2.31	0.517
			>29.0	1.72
			>29.0	0.935
P. vivax	2	2	4.16	0.403
			2.36	0.694
P. ovale	1	0	N/A	N/A
P. malariae	1	1	4.18	0.976

N/A = Not Applicable.

Table 3
Additional assessment of cross-reactivity due to DENV (n = 10), EBV (n = 10) or CMV (n = 10) infection.

Possible cross-reaction	n	LIAISON® XL Zika Capture IgM		
		POSITIVE (n)	ZIKV-M	ZIKV-C
DENV IgM	10	0	N/A	N/A
EBV IgM	10	5	2.40	0.782
			>29.0	0.775
			2.27	0.688
			2.71	0.642
			2.69	0.518
CMV IgM	10	6	2.41*	0.581
			2.75	0.707
			>29.0	0.772
			2.75	0.773
			>29.0*	0.601
			>29.0*	0.689

N/A = Not Applicable
* = sample obtained during pregnancy.

WA, USA) (82.8%) are higher but go at the cost of much lower specificity (Basile et al. 2018; L'Huillier et al. 2017; Safronetz et al. 2017).

IgM antibodies could already be detected with the LIAISON® XL Zika Capture IgM test as from day 2 pso. Other kits demonstrated low sensitivities up to 4 days (InBios), 5 days (Novatec) and 14 days (Euroimmun) pso (Basile et al. 2018; L'Huillier et al. 2017). During the first days after symptom onset, diagnosis of ZIKV infections should rely on PCR or in case PCR is not available, serology should be repeated on a convalescent sample.

One of the main challenges of serological tests is the immunological cross-reactivity with other anti-flavivirus antibodies (Lanciotti et al. 2008). This is especially important in areas with co-circulating flaviviruses. Because of the risk on adverse pregnancy outcome differentiation between ZIKV and other flaviviruses may be of utmost importance. We challenged the test with samples containing antibodies against DENV that is known to cocirculate with ZIKV, and *Plasmodium sp.* that is known to cause polyclonal stimulation resulting in false-positive reactions in the Euroimmun Anti-Zika virus ELISA used in our laboratory (Van Esbroeck et al. 2016), which is becoming a problem since more patients coming from the African continent are being screened for ZIKV. Cross-reactivity with DENV antibodies was limited (2/19, 10.5%) and restricted to serum samples from patients with a very recent DENV infection, as demonstrated by the presence of only IgM and no IgG antibodies and the presence of DENV RNA in one of both samples. The cross-reactivity due to DENV antibodies in our study was lower than the one reported in Canadian travelers (4/9, 44.4%) by Sloan et al. (Lustig et al. 2017).

The higher specificity of NS1 antigen based tests towards DENV was demonstrated before (Huzly et al. 2016; Steinhagen et al. 2016; Van Esbroeck et al. 2016) and is a major advantage in comparison with E-antigen based tests. In this study, cross-reaction in samples from patients with *Plasmodium sp.* infections was important (7/9, 77.8%). Surprisingly, some samples demonstrated cross-reaction with malaria when tested with the LIAISON® XL Zika Capture IgM test and not with the Euroimmun IgM ELISA and vice versa (data not shown). We demonstrated possible cross-reaction in samples containing IgM antibodies against EBV (6/13, 46.2%) and CMV (8/12, 66.7%), two other pathogens known to cause polyclonal stimulation. This observation which has not been reported before should further be examined, in particular since some of the presumably false-positive ZIKV results occurred in pregnant women with a confirmed CMV infection and both ZIKV and CMV can have serious implications during pregnancy. Presence of rheumatoid factor did not interfere in our study.

After this evaluation was finished, the manufacturer informed about a product formulation change intended to increase the specificity in both non-endemic and endemic populations, including patients with malaria. This second generation test was not evaluated in this study.

Our study suffered from some limitations. The patient cohort used mainly consisted of travelers, of which the majority is expected to have no flavivirus background apart from vaccinations against mainly YFV and to a lesser extent TBE or JEV which makes this an easier population to diagnose ZIKV infections. In addition we did not have the opportunity to include samples from pregnant women and the performance of the test in this patient group could therefore not be assessed. Further assessments of the CLIA test are needed with samples from residents of ZIKV endemic regions where other flaviviruses co-circulate and samples from residing pregnant women. Another limitation is that no samples from more than 84 days pso were included; therefore, the sensitivity of the test in samples beyond this date could not be established.

The major disadvantage of the test is that the system does not report ZIKV IgG antibodies. Knowing that IgM antibodies are only detectable up till 2–3 months, combination with IgG antibody detection is crucial for pregnant women, who traveled to ZIKV endemic areas and want to know if they were infected during pregnancy.

In conclusion, the high sensitivity even in early samples in combination with the limited cross-reaction with DENV antibodies makes the LIAISON® XL Zika Capture IgM test a reliable test in travel medicine. However, when used in patients coming from malaria endemic areas, mainly the African continent, caution is needed as cross-reaction with samples from patients with malaria is high. Also the cross-reaction with CMV and EBV is possibly high, which would be a major drawback especially when dealing with pregnant woman.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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