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Evaluation of A Novel IgM Screening Enzyme-Linked Immunosorbent Assay for Sensitive Detection of Mayaro Virus-Infected Patients

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ABSTRACT

Mayaro virus (MAYV) causes a febrile illness which is often associated with persistent arthralgia and can be clinically indistinguishable from other arbovirus infections. Serological differentiation is complicated by antibody cross-reactivity among alphaviruses. As the number of persons at risk of MAYV infection is increasing, there is a need for standardized laboratory assays enabling monitoring and diagnostic confirmation of MAYV infections. Here, the performance of the novel Euroimmun Anti-Mayaro Virus ELISA IgM was analyzed using sera from Brazilian patients with febrile infections diagnosed by in-house IgM antibody capture ELISA and indirect hemagglutination inhibition assay. In a panel comprising 21 MAYV positive patients and 25 MAYV negative controls, the Anti-Mayaro Virus ELISA IgM was 100% (21/21) sensitive and 76% (19/25) specific. Discrepant results were obtained in 6 samples from patients infected with chikunguya virus (CHIKV, 5/6) or flavivirus (1/6). The majority of deviations from in-house assays were likely due to cross-reactivity with the closely related CHIKV. In conclusion, the novel ELISA provides high sensitivity, enabling effective screening for MAYV-infected patients.

Keywords

Mayaro virus, Alphavirus, Arbovirus, ELISA.

Introduction

Mayaro virus (MAYV) is a member of the Alphavirus genus, family Togaviridae. It was first isolated from sick forest workers in Trinidad and Tobago (Caribbean) in 1954 [1]. Since then, sporadic outbreaks and infections of travelers have been recorded mainly in rural areas of northern South America (e.g. Brazil, Peru, Venezuela, and French Guiana) [2,3]. In 2016, MAYV was isolated from a child co-infected with dengue virus (DENV) in Haiti, suggesting re-emergence of MAYV in the Caribbean [4]. MAYV is transmitted from infected vertebrate hosts to humans usually by the bite of infected Haemagogus mosquitos [5]. Infection results in a nonfatal, self-limiting disease presenting with fever, chills, headache, rash, myalgia and, in more than 50% of patients, persistent incapacitating arthralgia [3,6-8]. As these manifestations can easily be confused with those of DENV, chikungunya virus (CHIKV), and Zika virus (ZIKV) infections, the clinical diagnosis of acute febrile illness in the context of different co-circulating arboviruses is difficult and prone to mistakes. This relates in particular to early disease stages when non-specific signs and symptoms are predominant, and to cases of co-infection, reflecting the need for laboratory confirmation [9]. Virus isolation and nucleic acid amplification techniques enable MAYV detection only during viremia in the first few days of illness. Afterwards, the diagnosis is often confirmed by serological testing [10-12]. However, only few standardized serological assays are available, and diagnosis may be complicated by antibody cross-reactions among alphaviruses [11,13].

There is growing concern that MAYV may expand on a global scale, similar to the recent CHIKV epidemics. Climate change, growing international travel, as well as deforestation, urbanization, and demographic changes in areas representing MAYV enzootic transmission foci have led to an increase in the number of persons at risk of MAYV infection [3,14]. In addition, there is evidence that MAYV can adapt to compatible urban vectors (Aedes spp.) [15]. Consequently, standardized laboratory assays are essential

for monitoring and diagnosis of MAYV infections. Here, we evaluated the analytical performance of a novel ELISA for the detection of anti-MAYV IgM in comparison with the well-established composite standard applied at the reference institute for arthropod-borne infections in Brazil.

Materials and Methods

Human serum samples

The study included 46 serum samples from Brazilian patients with febrile illness, which were collected 11-90 days after the onset of symptoms and sent for serological testing at the Instituto Evandro Chagas (Ananindeua, Pará, Brazil). Diagnostic criteria included clinical and epidemiological data. All MAYV samples were collected before CHIKV spread to Brazil. The CHIKV samples were taken during isolated outbreaks in periods without circulating MAYV, with infection confirmed by positive CHIKV RT-PCR. All samples were examined for IgM antibodies against MAYV, CHIKV, DENV, ZIKV, yellow fever virus (YFV), Oropouche virus (OROV), and flaviviruses in general using in-house IgM antibody capture (MAC) ELISA.

In addition, indirect hemagglutination inhibition (HI) testing was conducted to detect HI antibodies against MAYV, CHIKV, flaviviruses, eastern equine encephalomyelitis virus (EEEV), western equine encephalomyelitis virus (WEEV), and OROV. All samples from virus-infected patients showed a monotypic response using the HI assay. Based on composite MAC and HI antibody testing, the panel contained 21 sera from patients with MAYV infection, 17 from patients infected with other viruses (11 CHIKV, 2 DENV, 3 flavivirus, 1 OROV), and 8 seronegative cases. All anti-MAYV negative samples (according to the composite reference standard) will be referred to as controls. Individual and ethical approval was not mandatory for this study as samples were used anonymously.

Anti-Mayaro Virus ELISA IgM

All samples were analyzed using the Anti-Mayaro Virus ELISA IgM (Euroimmun, Lübeck, Germany). This assay is based on microplates coated with recombinant structural protein of MAYV. Testing was performed according to the manufacturer's instructions at the Instituto Evandro Chagas. Ratios were determined by dividing the extinction of the respective sample by the extinction of the assay calibrator. Ratio values <0.8 were considered negative, ≥0.8 to <1.1 borderline, and ≥1.1 positive for the presence of anti-MAYV IgM antibodies.

Statistical analysis

Statistics were performed using GraphPad QuickCalcs (GraphPad Software Inc., La Jolla, CA, USA) and SigmaPlot 13.0 (SSI, San Jose, CA, USA). Sensitivity was calculated as the proportion of MAYV samples identified as anti-MAYV IgM positive. Specificity was calculated as the proportion of negative/borderline anti-MAYV IgM results among control samples. Confidence intervals (95% CI) were calculated according to the modified Wald method. The t-test was used to analyze the difference in IgM ratios between the groups with P<0.05 considered significant.

Results

Compared with the composite standard of the reference institute, the IgM ELISA demonstrated positivity in all MAYV samples (21/21), corresponding to a sensitivity of 100%. Assay specificity amounted to 76.0% (19/25). Among controls, anti-MAYV IgM was detected in sera from 5 patients with CHIKV infection and in 1 sample positive for IgM against more than one flavivirus (Table 1, Figure 1). The mean IgM ratio in these 6 control samples was significantly lower than in the group of MAYV-infected patients (1.987 versus 6.248; t-test, P<0.001).

	Anti-Mayaro Virus ELISA IgM		
	N	Positive	Negative
MAYV	21	21	0
Sensitivity (CI 95%)	21	100% (81.8-100%)	
CHIKV	11	5	6
DENV	2	0	2
Flavivirus[a]	3	1	2
OROV	1	0	1
Negative	8	0	8[b]
Specificity (CI 95%)[c]	25	76.0% (56.3-88.8%)	

Table 1: Assay performance.
CHIKV: chikungunya virus; CI: Confidence interval; DENV: dengue virus; MAYV: Mayaro virus; OROV: Oropouche virus.
[a]Samples categorized as general flavivirus infection due to crossing with ≥ 2 flaviviruses.
[b]Including one sample with borderline reactivity.
[c]For the calculation of specificity, borderline results were considered negative.

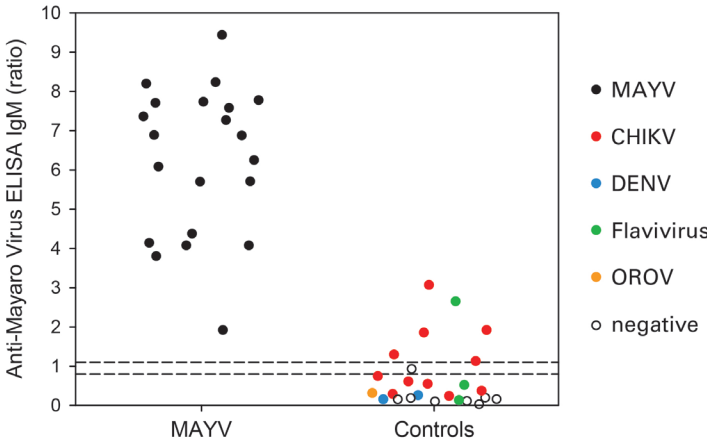

Figure 1: Determination of anti-MAYV IgM in MAYV-infected patients and controls. Dashed horizontal lines represent the cut-off ratios for borderline and positive results.

Discussion

Overall, the Euroimmun Anti-Mayaro Virus ELISA IgM demonstrated high sensitivity at moderate specificity. False-positive reactivity in some sera from CHIKV-infected patients is most likely explained by the presence of cross-reactive anti-CHIKV antibodies [16]. MAYV and CHIKV are alphaviruses belonging to the Semliki Forest serocomplex, which also includes Bebaru virus, getah virus, o'nyong-nyong virus (ONNV), Ross

River virus (RRV), Semliki Forest virus, and Una virus [17]. The nucleocapsid protein of alphaviruses contains group-reactive antigenic determinants, whereas the envelope proteins E1 and E2 display multiple complex-reactive and species-specific epitopes [11,18,19]. In line with this, Calisher et al. reported cross-reactivity of IgM in sera from CHIKV-infected patients with MAYV, ONNV, and RRV using MAC ELISA [13]. Given the similarity of clinical symptoms and the overlapping geographic distribution of MAYV and CHIKV in tropical South America and the Caribbean, cross-reactivity and the possibility of co-infection should be considered in differential serodiagnostics. Species-specific neutralization tests may help to correctly determine the causative virus.

Other types of interference cannot be excluded. This is supported by the fact that one false-positive anti-MAYV IgM result was obtained in a sample showing IgM cross-reactivity between several flaviviruses. In fact, many Brazilians have a history of multiple arboviral infections, which may increase the complexity of samples.

Notably, antibody ratios measured in IgM false-positive sera were all very low and in part only marginally above the cut-off for positivity (Figure 1). As anti-MAYV IgM ratios among true-positive samples were on average 4 ratio units higher, low-positive IgM results appear to result more frequently from interferences, causing false positivity. Placing the IgM cut-off above ratio 3.1 would result in a sensitivity of 95% and a specificity of 100% in this sample set.

IgM testing would be complemented by the additional determination of anti-MAYV IgG, preferably using paired samples to assess seroconversion. In a panel comprising 6 MAYV-infected patients and 6 MAYV negative controls, the Anti-Mayaro Virus IgG ELISA revealed a sensitivity of 100% (6/6), while specificity amounted to 50% (3/6) due to positivity in 3 CHIKV cases. However, these are only preliminary data collected in an insufficient number of cases.

Our findings are subject to several limitations. Firstly, as this was a pilot study, only a relatively small sample size was examined. This may have led to under- or overestimates of assay accuracy, as reflected by wide confidence intervals for sensitivity and specificity calculations. Secondly, sample pre-characterization included clinical, epidemiological, HI and MAC data, whereas virus neutralization was not tested. Thirdly, we did not perform systemic analysis of ELISA specificity, lacking a sufficiently high number of sera representing infections with (or vaccinations against) other viruses relevant in differential diagnostics, and in particular healthy controls. Finally, the study did not address characteristics of IgM and IgG responses to primary and secondary infections with MAYV and other alphaviruses, which is highly relevant in endemic settings [20].

Conclusion

The highly sensitive Euroimmun Anti-Mayaro Virus ELISA IgM is an efficient screening tool for serodiagnostic and epidemiologic surveillance purposes, extending the time window for detecting acute and recent MAYV infections beyond the viremic phase. It can be applied by any standard laboratory and offers a standardized, simple, fast, and cost-effective alternative to other techniques. Users of this assay should be aware of possible interferences due to cross-reactivity with antibodies against closely related alphaviruses.

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