Journal of Virological Methods 260 (2018) 26–33

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Multi-laboratory comparison of three commercially available Zika IgM enzyme-linked immunosorbent assays

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ARTICLE INFO

Keywords:
Zika
Dengue
Virus
Kits
ELISA
Enzyme-linked immunosorbent assay
Commercial
IgM
IgG
Immunoglobulin M
Immunoglobulin G
Comparison
Nonstructural protein 1
NS1
Envelope E

1. Introduction

Zika virus (ZIKV) is an enveloped, positive-sense RNA virus in the family *Flaviviridae*, genus *Flavivirus*. It was first discovered in rhesus monkeys in 1947 in the Zika Forest of Uganda (Dick et al., 1952) and historically of unclear importance given the rarity of reported cases and to relatively mild symptoms in humans. The virus is chiefly transmitted by *Aedes* mosquitoes, the carrier of other flaviviruses of medical importance such as the dengue viruses (DENVs) and yellow fever virus (YFV). Little research had been conducted on ZIKV prior to a 2007 outbreak in Yap, Federated States of Micronesia (Duffy et al., 2009), at which point the virus was sequenced and molecular and serological tests were developed (Lanciotti et al., 2008).

Zika virus was identified in French Polynesia in 2013 (Cao Lormeau et al., 2014), and Easter Island in 2014 (Tognarelli et al., 2016). Following this, an extensive epidemic began in late 2015 first identified in Natal, northeastern Brazil (Zanluca et al., 2015). The virus subsequently spread widely among Central/South America and the Caribbean (Wikan and Smith, 2016), with small clusters of local disease transmission identified in the United States in Florida and Texas (Khawar et al., 2017). Sequencing identified that the ZIKV currently circulating in the Americas is derived from the Southeast Asian genotype (Brasil et al., 2016). The discovery of increased incidence of microcephaly and other birth defects in newborns (Fitzgerald et al., 2018) took ZIKV from being considered as relatively benign to being a critical public health concern, causing the World Health Organization (WHO) to declare a public

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https://doi.org/10.1016/j.jviromet.2018.06.018
Received 6 March 2018; Received in revised form 6 June 2018; Accepted 28 June 2018
Available online 30 June 2018

health emergency in 2016 (Gulland, 2016).

The CDC Zika IgM-antibody capture enzyme-linked immunosorbent assay (Centers for Disease Control and Prevention Zika MAC-ELISA) was developed for detection of ZIKV immunoglobulin M (IgM) at the CDC in 2007 (Lanciotti et al., 2008), and was recognized as highly cross-reactive with other members of the flavivirus genus. The CDC Zika MAC-ELISA, utilizing inactivated whole virus ZIKV antigen, was used for serological diagnosis during the Yap outbreak in combination with reverse-transcriptase polymerase chain reaction (RT-PCR) in acute samples. The CDC Zika MAC-ELISA was granted Emergency Use Authorization (EUA) by the US Food and Drug Administration because of the need for widespread use in testing of samples from returning US travelers, and later in the outbreak, for use in the diagnosis of autochthonous cases.

Before 2016, commercial serologic test kits for ZIKV were absent. In the latter part of that year, three commercial ZIKV IgM ELISAs became available, one of which was emergency use authorized. The purpose of this study was to evaluate these kits in comparison to the reference diagnostic results, using a combination of ELISA and 90% plaque-reduction neutralization test (PRNT90) results. We aimed to determine sensitivity, specificity, and consistency of results across three geographically separated labs, using well-characterized blinded sera.

2. Materials and methods

2.1. Laboratories

Three laboratories participated in the study: Arbovirus Diseases Branch – Diagnostic and Reference Laboratory (ADB-DRL), CDC, Fort Collins, CO; Microbial Pathogenesis and Immune Response (MPIR) Laboratory, CDC, Atlanta, GA; Public Health Agency of Canada (PHAC), National Microbiology Laboratory, Winnipeg, Canada. All three participating laboratories were previously shown to be proficient in the use of the CDC Zika MAC-ELISA through participation in the CDC Arbovirus Proficiency Program. The ADB-DRL and PHAC laboratories function as arbovirus reference laboratories for the US and Canada, respectively.

2.2. Reference assays

Reference data were generated at the ADB-DRL for the serum panels described in Section 2.4 through a combination of CDC Zika MAC-ELISA and CDC DENV MAC-ELISA results (Lindsey et al., 2018), and confirmed by PRNT90 (Beaty et al., 1995) according to the CDC diagnostic algorithm https://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf. The West Nile virus (WNV, family *Flaviviridae*, genus *Flavivirus*) IgM-positive, YFV (family *Flaviviridae*, genus *Flavivirus*) IgM-positive and chikungunya virus (CHIKV, family *Togaviridae*, genus *Alphavirus*) IgM-positive samples were tested by CDC MAC-ELISA and confirmed by PRNT90 for the respective viruses, and Zika and DENV MAC-ELISA results were also generated for these samples.

Inactivated whole ZIKV antigen generated in Vero E6 cells was used in the EUA Zika MAC-ELISA, and a combination of recombinant antigens of DENV serotypes 1–4 (E/prM proteins) made in COS-1 cells (Russell et al., 2007) was used in the DENV MAC-ELISA. Flavivirus group-reactive monoclonal antibody 6B6C-1-horseradish peroxidase (Tsai et al., 1987), custom-conjugated for the CDC by Jackson Immunoresearch (West Grove, PA), was used to detect reactions in both the Zika and DENV MAC-ELISAs.

The arbovirus MAC-ELISAs are qualitative tests, and the P/N ratio (optical density (OD) of the sample reacted on viral antigen/OD of negative control reacted on viral antigen) is not intended to compare results across samples. A P/N value of ≥ 3.0 is considered presumptive positive IgM; < 2.0 is considered negative, and results in between these values are considered equivocal. Positive, equivocal, and uninterpretable CDC Zika MAC-ELISA results were confirmed by PRNT90 using strain ZIKV strain PRVABC59, DENV serotype 1 (ChimeriVax YF/DEN chimera), and/or DENV serotype 2 (ChimeriVax YF/DEN chimera) (Guirakhoo et al., 2000). In the PRNT90, a sample was considered negative for neutralizing antibody to the challenge virus when 90% plaque-reduction was not observed at the lowest serum dilution used (1:10).

2.3. Kits

Kits from three manufacturers were used for the comparison. 1) ZIKV Detect™ IgM Capture ELISA (InBios International Inc., Seattle, WA) uses a recombinant ZIKV antigen made to the E/prM proteins, and also a cross-reactivity control antigen (CCA) made using recombinant DENV (Russell et al., 2007) and WNV prM/E proteins (Davis et al., 2001). This assay gained EUA in September, 2016. 2) NovaLisa® Zika IgM μ-capture ELISA (NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) uses ZIKV nonstructural protein 1 (NS1) antigen. 3) The combined assays Anti-Zika Virus ELISA (IgM) and Anti-Zika Virus ELISA (IgG) (Euroimmun Medizinische Labordiagnostika AG (Lübeck, Germany), both use ZIKV NS1 antigen. For brevity in this paper, the kits are generally referred to as follows: InBios ZIKV Detect™ Capture IgM ELISA "InBios kit"; NovaTec NovaLisa® Zika IgM μ-capture ELISA "NovaTec kit"; Euroimmun Anti-Zika Virus (IgM) "Euroimmun IgM kit"; Euroimmun Anti-Zika Virus (IgG) "Euroimmun IgG kit"; and when used together, the Euroimmun IgM and IgG kits are referred to as "Euroimmun IgM + IgG kits". The respective manufacturers generously provided all kits for this study.

Kit result interpretations varied according to manufacturer. Briefly, the InBios kit had four outcome categories: presumptive Zika positive, possible Zika positive, presumptive other flavivirus positive, and negative. The NovaTec kit had three outcome categories: positive, equivocal and negative. Euroimmun IgM and IgG kits each had three outcome categories: positive, borderline, and negative.

Kits were received at all three laboratories directly from the manufacturers in good condition, and each participating lab received the same lot number of kits from the individual manufacturers, with the exception of those from InBios, where it was necessary to obtain additional kits at a later date due to QC failures on using the first lot. Kits were stored at 4 °C prior to use, and used within a month of receipt and were well within the expiry dates. Results were classified according to the individual kit instructions and shared with the manufacturers. The Euroimmun IgM and IgG kit results were combined per the recommendation of Euroimmun, whereby a sample that gave positive results in either or both of the assays was classified as positive. Repeat testing of samples with equivocal or borderline results was not performed. Additional kits of an alternate lot number were obtained from NovaTec for purposes of testing Panels 2 and 3 (see Sections 2.4.2 and 2.4.3), and testing was performed at the ADB-DRL only for these panels.

All samples from Panel 1 (see Section 2.4.1) were tested using each kit at each of the three laboratories by adhering to the manufacturer's instructions. Where options existed in the manufacturer's instructions, all three labs agreed upon which option to use. For the NovaTec kit, the results acquisition option of using a reference wavelength of 630 nm was chosen. For the Euroimmun IgG kit, the quantitative results calculation option was used. Samples were run singly with all test kits. Plate validity parameters passed quality control prior to including results in the study, and plates were repeated as necessary to obtain valid results. Kits were subjectively assessed for ease-of-use, and the comparative features of each kit are presented in Table 1.

2.4. Serum samples

Panels were prepared in accordance with CDC Institutional Review Board protocol #6773 "Use of human specimens for laboratory research on arboviruses".