Abstract

There is lack of accurate serological diagnosis of the Zika virus due to cross-reactivity with other flaviviruses. We have developed an Anti-Zika IgG test that is highly sensitive to Zika virus and displays minimal cross-reactivity with dengue virus IgG. R&D Systems® Anti-Zika Virus IgG test will potentially allow for a highly specific serological test for Zika virus infection.

Introduction

Zika virus (ZIKV) is a mosquito-borne flavivirus found throughout tropical and subtropical regions, including East Africa, Southeast Asia, and the Pacific Islands, that is now causing large-scale outbreaks in the Americas.2,3 This continuous geographic expansion of ZIKV poses a serious and increasing public health threat around the globe.4,5 Initially, ZIKV infection was thought to cause only mild illness, however it has now been linked to a rising number of severe neurological diseases including microcephaly, congenital abnormalities, and nonfetal illnesses such as Guillain-Barré syndrome, which emphasizes the importance of accurate ZIKV diagnostics.2,4,5

Serological diagnosis is complicated by cross-reactivity among members of the Flavivirus genus.4 Because ZIKV and dengue virus (DENV) co-circulate in endemic regions and share high sequence similarity, there is a high possibility of IgM and IgG cross-reactivity in immunoassays.1 Current or past infections will often cause false positives requiring the need for follow-up testing and confirmation by a plaque-reduction neutralization (PRNT) assay. PRNT is a complicated method that takes considerable time and has limited availability.1,4 In addition, antibodies present from past infection by Zika or other flaviviruses may enhance the risk of future ZIKV infections through antibody-dependent enhancement (ADE), which may lead to increased disease severity.1 There is a need for a simple serological test that is specific for anti-Zika virus IgG and displays minimal dengue virus cross-reactivity.

We addressed this need by developing a highly specific anti-Zika virus IgG assay. We tested this kit alongside three commercially available anti-ZIKV IgG ELISA kits currently on the market. Using serum samples from patients diagnosed with Zika or dengue virus infections, we directly compared the sensitivity and specificity of each test. The R&D Systems assay is a highly specific and sensitive test.

Methods

Samples from groups of patients that were diagnosed with either ZIKV infections (collected from Colombia between 2015 and 2016), or DENV infections (collected from Puerto Rico between 2012 and 2013, prior to the introduction of Zika to Puerto Rico), were tested for the presence of anti-ZIKV IgGs using R&D Systems® Anti-Zika Virus IgG ELISA Kit, or three commercially available anti-ZIKV IgG ELISA kits. The same set of samples was tested with all of the kits. All tests were performed following the manufacturers' recommendations.

Results

Table 1. Position of the assay was tested to determine the reproducibility of results, within and between assays. Intra-assay CV was determined to be less than 2% and inter-assay CV was determined to be less than 12%.

Table 2. ELISA assay of Zika virus patient samples, Z18, Z20, Z35, and Z39, and Dengue virus patient samples, Z18, Z20, Z35, and Z39, tested both without (left graph) and with pretreatment of the samples (right graph). Because sample pre-treatment results in a specific assay, this data suggests that due to cross-reactivity, a positive test for anti-Zika virus IgG using Zika patient samples Z18, Z20, Z35, and Z39 is a false positive result.

Figure 3. R&D Systems® Anti-Dengue Virus IgG ELISA

Figure 4. Vendor A Anti-Zika IgG Test. One of the fifteen dengue virus patient samples that were analyzed tested positive for the Zika virus. The assay was performed following the manufacturer’s recommendations.

Figure 5. Vendor B Anti-Zika IgG Test. Only two of the fifty Zika virus patient samples that were analyzed tested positive for the Zika virus. One of the five dengue virus patient samples that were analyzed tested positive for the Zika virus. The assay was performed following the manufacturer’s recommendations.

Conclusions

Performance of R&D Systems® Anti-Zika Virus IgG ELISA:

- High sensitivity and specificity
- Very low cross-reactivity with dengue virus IgG

Three other commercially available anti-ZIKV IgG ELISA kits tested displayed either:

- High cross-reactivity with dengue virus IgG OR
- Low sensitivity to anti-Zika virus IgG

References