

Abstract

There is lack of accurate serological diagnosis of the Zika virus due to cross-reactivity with other flaviviruses. We tested three commercially available anti-ZIKV IgG ELISA kits currently on market. Contrary to the manufacturers' claims, we find that these kits have either high cross-reactivity with dengue virus or low Zika virus sensitivity.

We have developed an Anti-Zika IgG test that is highly sensitive to Zika and displays minimal cross-reactivity with anti-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.^{1,2} This continuous geographic expansion of ZIKV poses a serious and increasing public health threat around the globe.^{1,4} 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.^{2,4,6}

Serological diagnosis is complicated by cross-reactivity among members of the *Flavivirus* genus.⁶ 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.⁷ 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.^{7,8} 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.⁹ There is a need for a simple serological test that is specific for anti-Zika virus IgG and displays minimal dengue virus cross-reactivity.

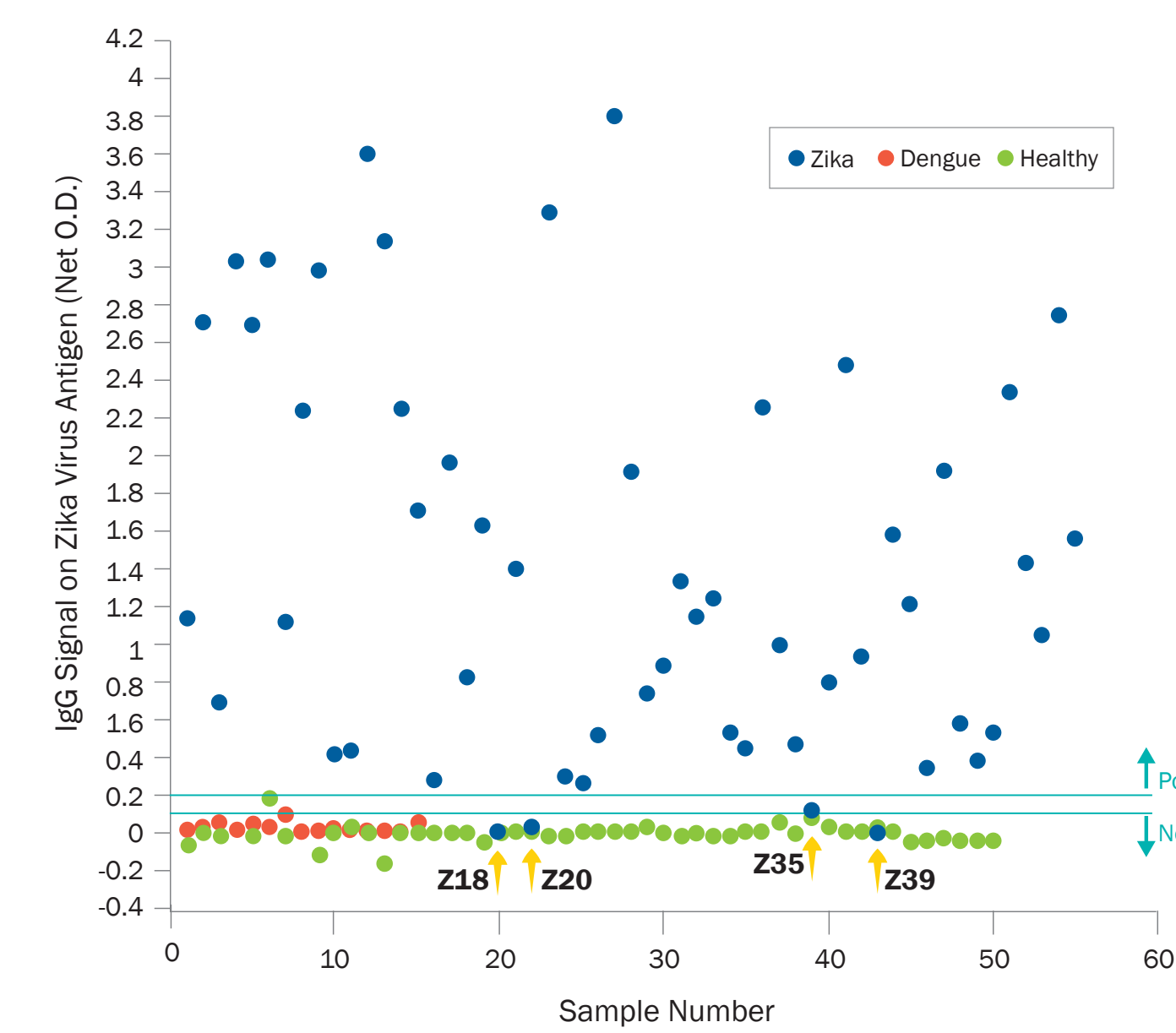
We addressed this need by developing a highly specific anti-Zika virus IgG assay. We tested this kit alongside three commercially available anti-ZIKV IgG ELISA kits currently on the market. Using serum samples from patients diagnosed with Zika or dengue virus infections, we directly compared the sensitivity 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

R&D Systems® Anti-Zika Virus IgG ELISA



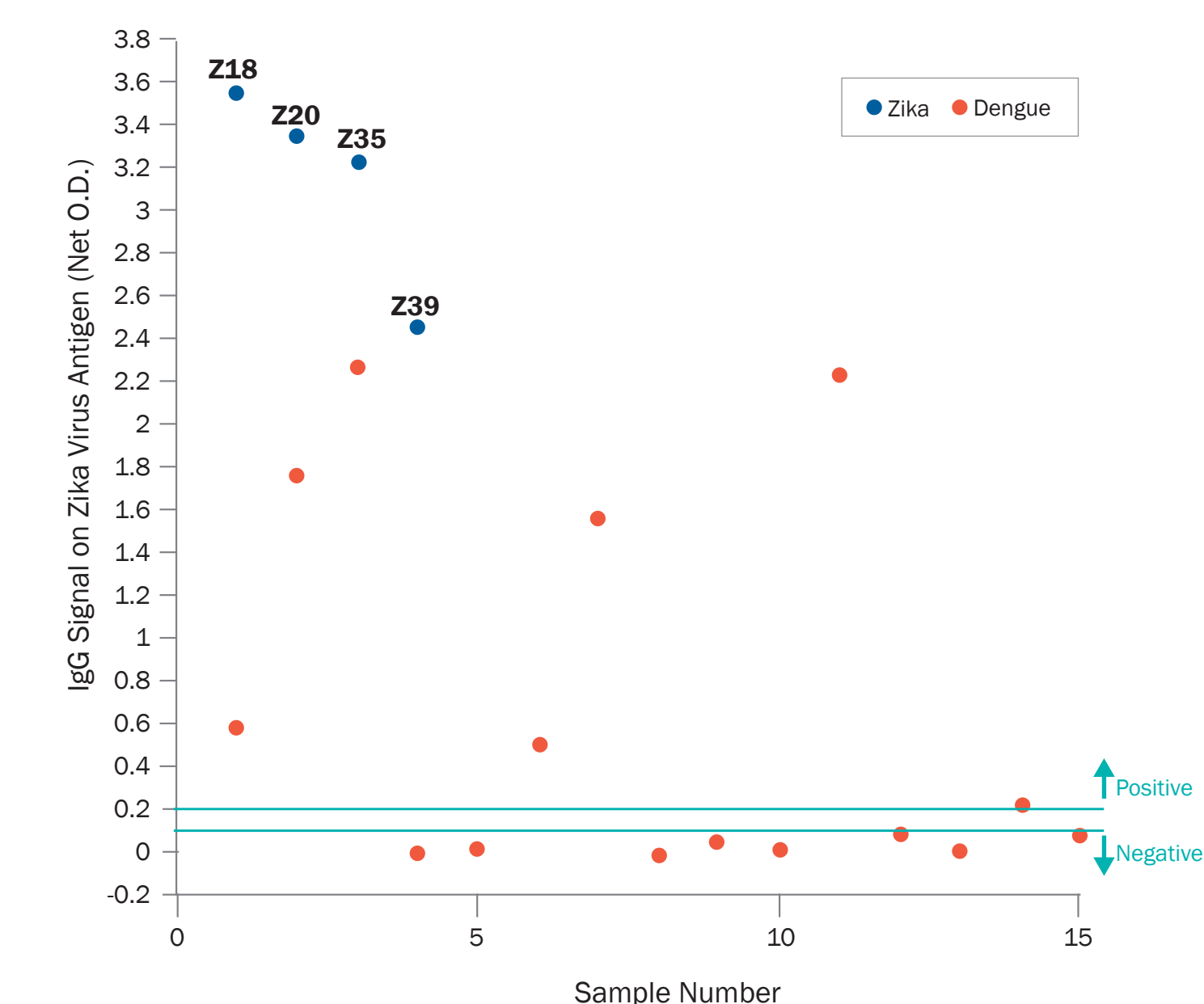
R&D Systems® Anti-ZIKV IgG ELISA is 100% specific.

Figure 1. R&D Systems® Anti-Zika Virus IgG Test. Using R&D Systems® Anti-Zika Virus IgG test, dengue patient samples (shown in red) have the same net O.D. as healthy donor samples (shown in green), which is similar to background. Fifty-five Zika patients (shown in blue) were tested, of which fifty-one were positive and four were negative (indicated by the yellow arrows). These four samples, Z18, Z20, Z35, and Z39, which were reported to be anti-Zika virus IgG positive by the supplier, are actually dengue positive samples and do not contain specific anti-Zika IgG antibodies (See Figures 2 and 3).

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	24	24	24	20	20	20
Net O.D.	0.597	1.08	1.94	0.616	0.992	1.81
Standard Deviation	0.021	0.056	0.139	0.058	0.113	0.168
CV (%)	3.5	5.2	7.2	9.4	11.4	9.3

Table 1. Precision of the assay was tested to determine the reproducibility of results, within and between assays. Intra-assay CV was determined to be less than 7.5% and inter-assay CV was determined to be less than 12%.

Without Sample Pre-Treatment



With Sample Pre-Treatment

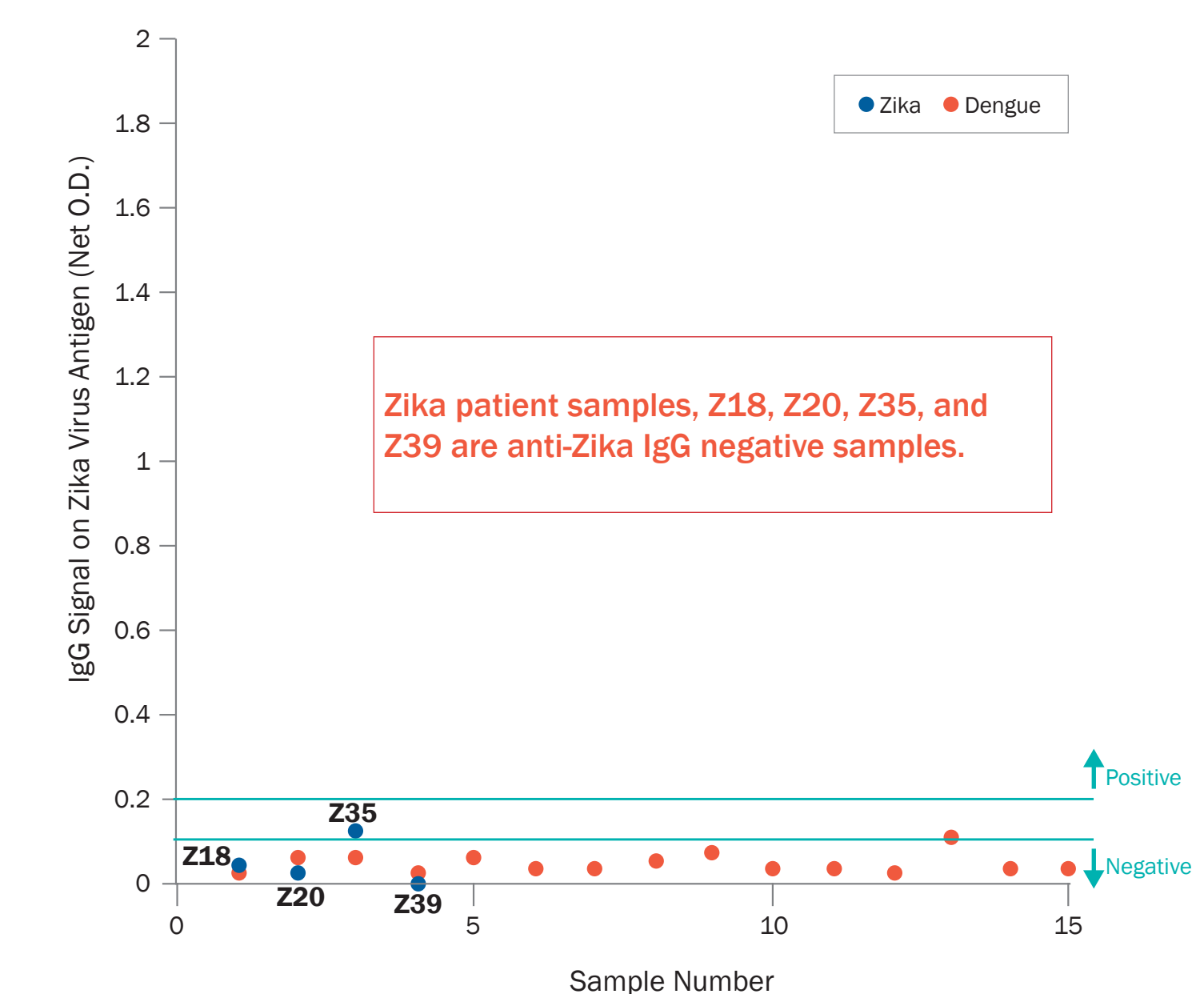
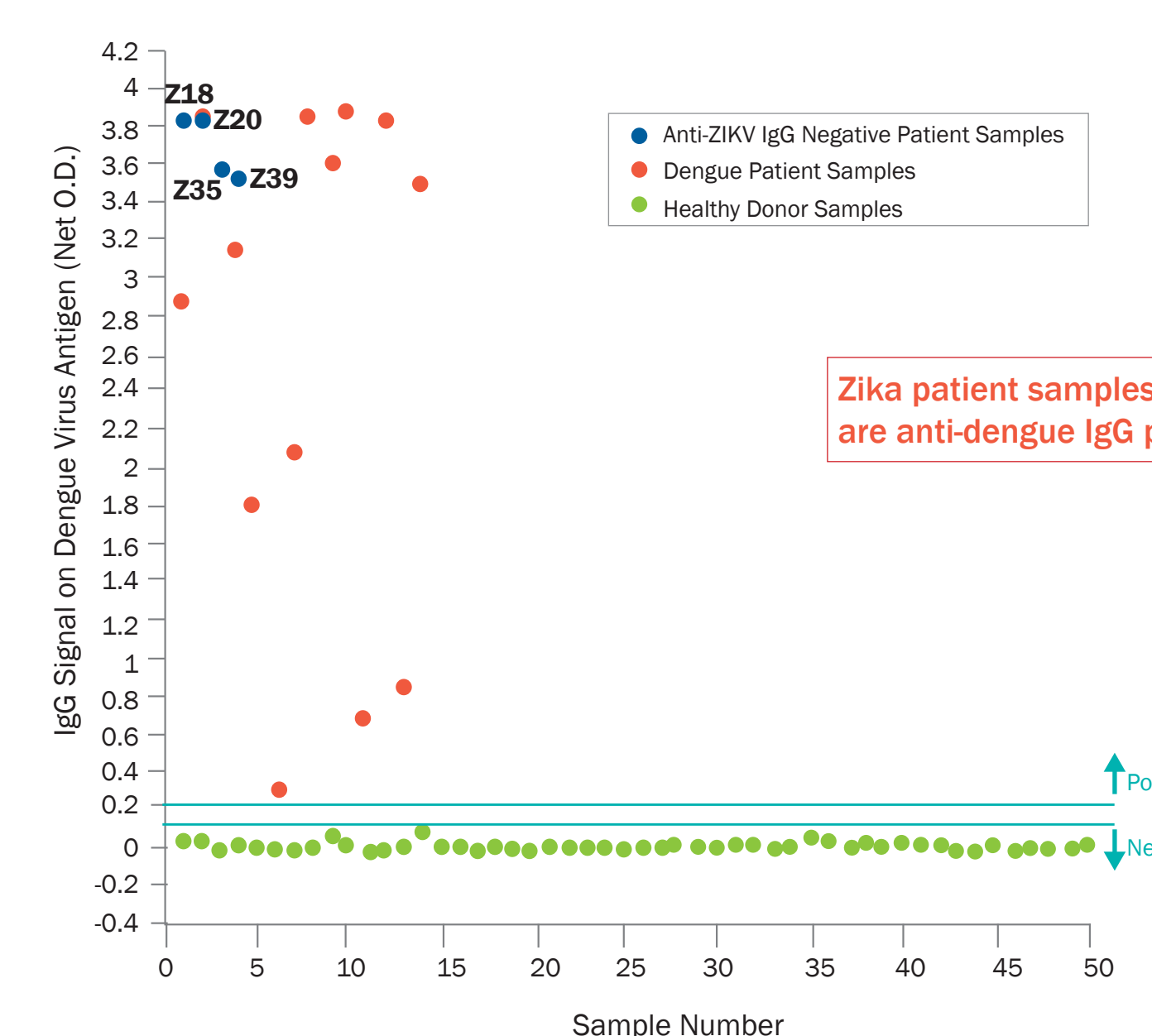


Figure 2. The four Zika virus patient samples, Z18, Z20, Z35, and Z39, and fifteen dengue virus positive patient samples were tested both without (left graph) and with pre-treatment of the samples (right graph). Because sample pre-treatment results in a specific assay, this data suggests that due to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

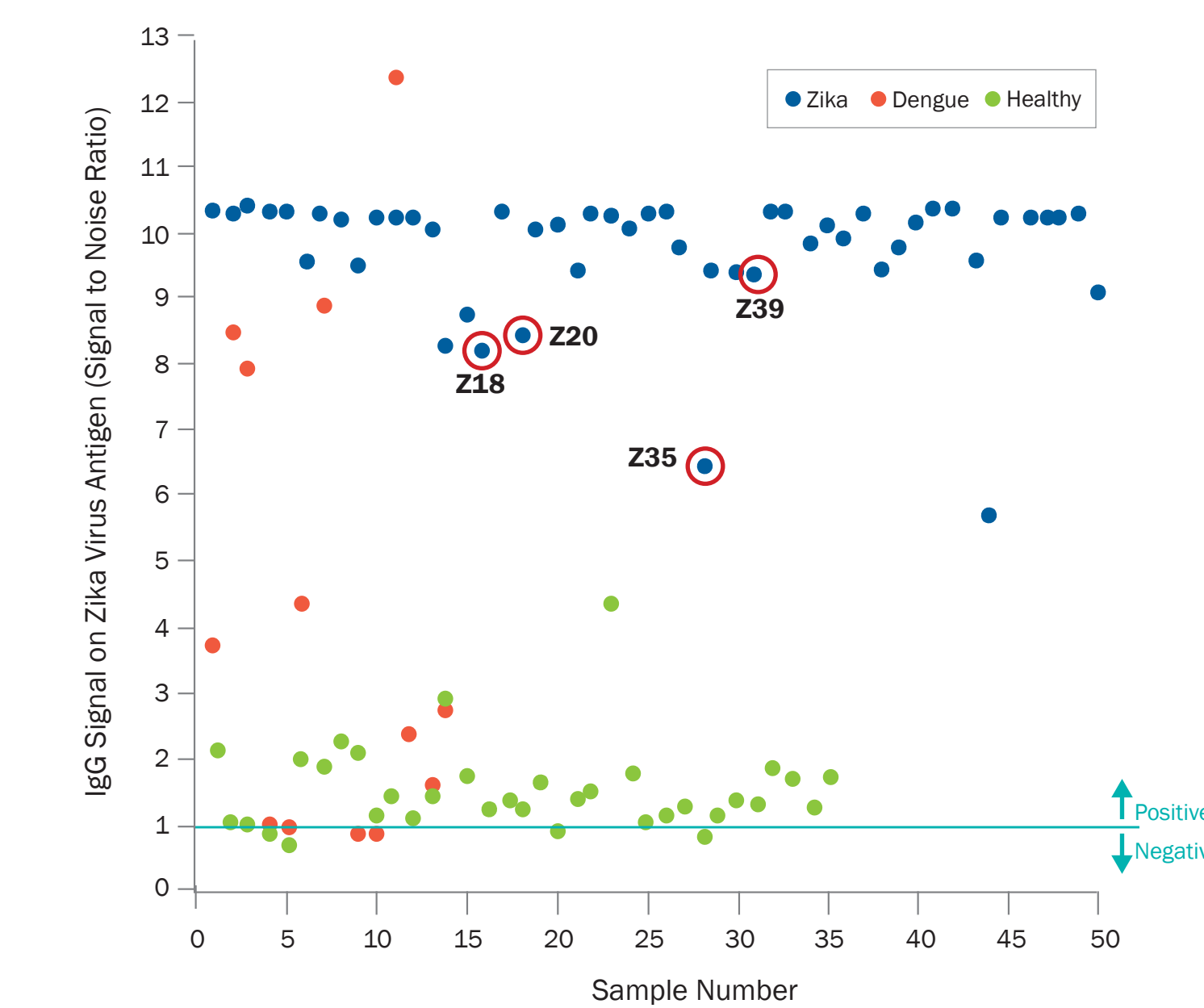
R&D Systems® Anti-Dengue Virus IgG ELISA



Zika patient samples, Z18, Z20, Z35, and Z39, are anti-dengue IgG positive samples

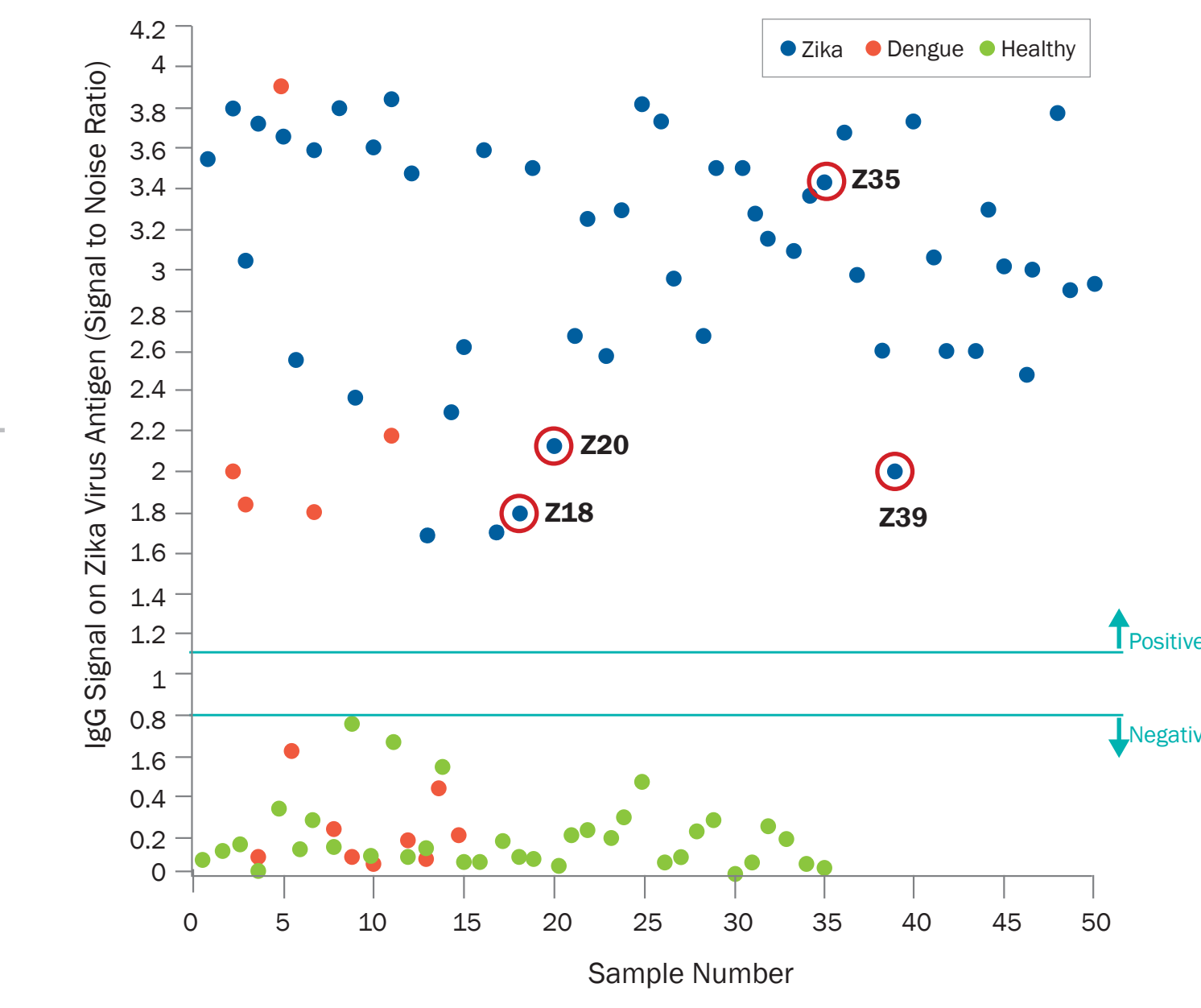
Figure 3. R&D Systems® Anti-Dengue IgG Test. Dengue patient and healthy donor samples were tested to determine thresholds, in a manner similar to our Anti-Zika IgG ELISA. We then used this assay to test Zika patient samples, Z18, Z20, Z35, and Z39. This assay is designed to treat samples with reagents formulated to create high specificity for dengue.

Testing of Other Commercially Available Anti-Zika Virus IgG ELISAs



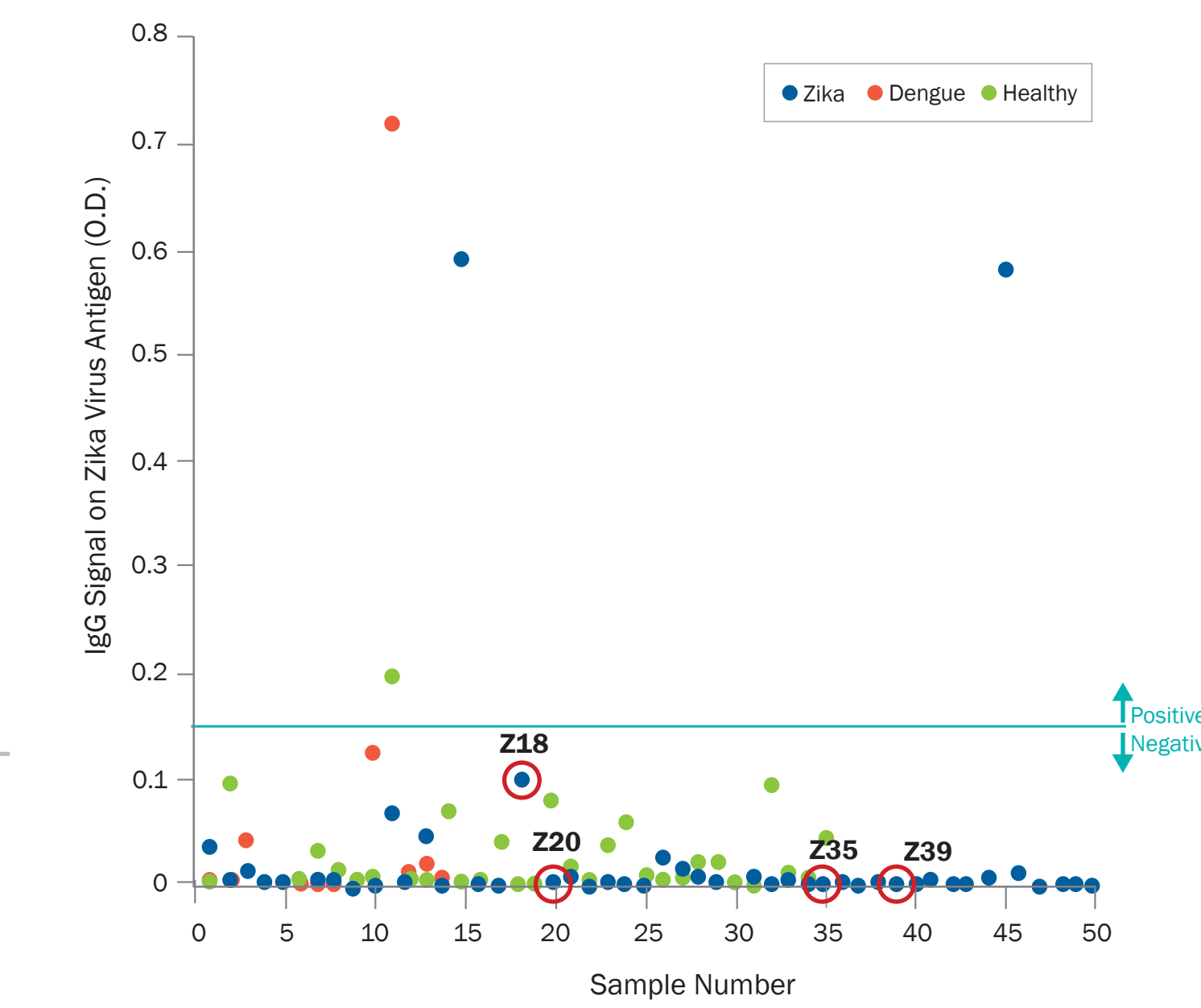
The assay from Vendor A shows ~70% cross-reactivity with dengue virus patient samples and also produced false positive results for 80% of the presumed healthy donor samples tested.

Figure 4. Vendor A Anti-ZIKV IgG Test. Eleven of the fifteen dengue virus patient samples that were analyzed tested positive for the Zika virus. Thirty of the thirty-five healthy patient samples that were analyzed, also tested positive for the Zika virus. Zika patients, Z18, Z20, Z35 and Z39, which we found do not contain Zika specific antibodies, also tested positive. The assay was performed following the manufacturer's recommendations.



The assay from Vendor E shows ~30% cross-reactivity with dengue virus patient samples.

Figure 5. Vendor E Anti-ZIKV IgG Test. Five of the fifteen dengue virus patient samples that were analyzed tested positive for the Zika virus. Zika patients, Z18, Z20, Z35, and Z39, which we found do not contain Zika specific antibodies, also tested positive. The assay was performed following the manufacturer's recommendations.



The assay from Vendor M had low sensitivity, with 96% of the 50 reported ZIKV positive samples producing negative results.

Figure 6. Vendor M Anti-ZIKV IgG Test. Only two of the fifty Zika virus patient samples that were analyzed tested positive for the Zika virus. 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.

Conclusions

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

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

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

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

References

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