# Supplementary Materials:

**Validation of flaviMIA**

A total of 897 positive samples and 70 negative sera were selected as a test validation panel.

Well characterised sera from RT-PCR-confirmed primary cases were selected. Because the specificity of IgM does not always reflect the current infecting serotype in secondary dengue cases, presence of RNA was used for sample selection rather than IgM detection.

Acute and convalescent sera are described separately. Sensitivity and specificity data for each component of the assay are listed in Supplementary Table 1. After optimisation, an arbitrary cut-off of 2000 MFI was chosen and sensitivity and specificity of the assay were compared to the PCR results. A ratio of 1.5 was set to determine the specificity of the antibody detected i.e. where the MFI output for antibody reactivity to one virus was 1.5 or more times higher than that signal to any other viruses in the panel, the signal was described as specific antibody reactivity to the first virus. This factor allowed 33.7% of all sera to be correctly assigned to a virus species (i.e. dengue serotype, ZIKV etc.), and a further 19.6% to be correctly assigned to a virus group (e.g. to DENV). The remainder of the sera were described as Flavivirus cross-reactive, or negative.

**Table S1.** Description of samples used to validate the flaviMIA.

| Sera | No. Positive sera | Reference assay | flaviMIA Sensitivity | flaviMIA Specificity |
|---|---|---|---|---|
| DENV-1 Acute | 270 | PCR | 44.8 | 97.1 |
| DENV-1 | 42 | PCR[1] | 100.0 | 97.1 |
| DENV-2 Acute | 161 | PCR | 49.1 | 97.1 |
| DENV-2 | 31 | PCR[1] | 100.0 | 97.1 |
| DENV-3 Acute | 106 | PCR | 47.2 | 98.6 |
| DENV-3 | 21 | PCR[1] | 95.2 | 98.6 |
| DENV-4 Acute | 59 | PCR | 45.8 | 95.7 |
| DENV-4 | 16 | PCR[1] | 93.8 | 95.7 |
| JEV | 29 | UCHI/HAI | 93.1 | 100.0 |
| MVEV | 3 | PCR, clinical | 66.7 | 100.0 |
| KUNV | 41 | UCHI/HAI | 70.7 | 100.0 |
| ALFV | 0 | Samples not available | Samples not | Samples not |
| KOKV | 27 | UCHI/HAI | 81.5 | 100.0 |
| STRV | 0 | Samples not available | Samples not | Samples not |
| YFV | 28 | Post-vaccination sera, | 92.9 | 98.6 |
| ZIKV Acute | 38 | PCR, PRNT | 47.4 | 100.0 |
| ZIKV | 25 | PCR*, PRNT | 100.0 | 100.0 |

[1]RNA detected in acute sample from same patient; UCHI – ultracentrifugation-hemagglutination inhibition; HAI – hemagglutination inhibition assay

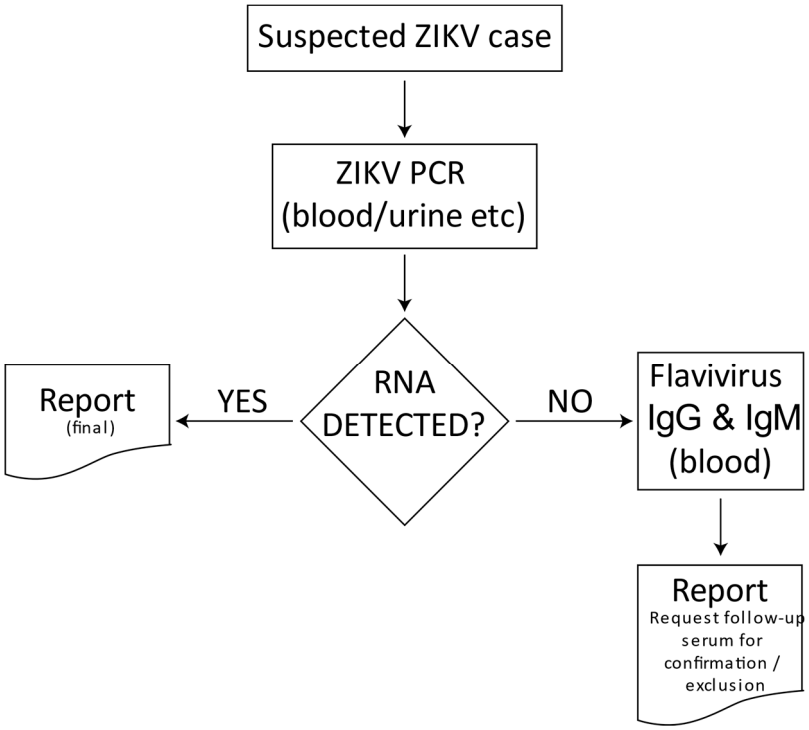


**Figure S1**. Comprehensive testing algorithm for ZIKV requests.
When specifically requested, a nucleic acid extract from sera is screened for ZIKV RNA using two previously described Asian-lineage specific real-time RT-PCR methods.{6095}
Specimens from which ZIKV RNA is not detected are examined for the presence of flavivirus antibodies following the process outlined in Figure 1.