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*Table S1: Molecular tests used for the diagnosis of DENV/ZIKV infection. The genus species group, the target gene, the type of the oligo, the oligo IS, the 5'-3' sequence, the expected PCR fragment in bp and references are provided.*

| Genus species group | Target gene         | Oligo type       | Oligo ID      | Sequence (5'-3')                         | PCR fragment (bp) | Reference |
|---------------------|---------------------|------------------|---------------|--|-------------------|-----------|
| DENV                | 3' noncoding region | fwd <sup>a</sup> | DenS          | GGATAGACCAGAGATCCTGCTG T                 | 79                | [50]      |
|                     |                     | rev <sup>b</sup> | DenAs         | CATTCCATTTTCTGGCGTTC                     |                   |           |
|                     |                     | rev              | DenAs1        | CAATCCATCTTGCGGCGCTC                     |                   |           |
|                     |                     | probe            | DenP          | FAM-CAGCATCATTCCAGGCACAG-TAMRA           |                   |           |
|                     |                     |                  |               |  |                   |           |
| ZIKV                | envelope E protein  | fwd              | Zvf1086       | CCGCTGCCCCAACACAAG                       | 53                | [51]      |
|                     |                     | rev              | ZIKV 1162c    | CCACTAACGTTCTTTGCAGACA T                 |                   |           |
|                     |                     | probe            | ZIKV 1107-FAM | FAM-AGCCTACCTTGACAAGCAGTCA GACTCAA-TAMRA |                   |           |
|                     |                     |                  |               |  |                   |           |
| Triplex             | DENV 5'UTR          | fwd              | DENV-F        | TAGTCTRCGTGGACCGACAAG                    |                   |           |

|                     |               |       |         |  |  |                                    |
|---------------------|---------------|-------|---------|--|--|------------------------------------|
| (DENV, ZIKV, CHIKV) |               |       |         |  |  |                                    |
|                     |               | rev   | DENV-R1 | CAGTTGACACRCGGTTTCTC                             |  |                                    |
|                     |               | rev   | DENV-R2 | GGGTTGATACGCGGTTTCTC                             |  |                                    |
|                     |               | probe | DENV-P  | FAM-CGYCTWTCAATATGCTGAAACG<br>CG-BHQ1            |  |                                    |
|                     | CHIKV nsp1    | fwd   | CHIKV-F | ACCATCGGTGTTCCATCTAAAG                           |  |                                    |
|                     |               | rev   | CHIKV-R | GCCTGGGCTCATCGTTATT                              |  |                                    |
|                     |               | probe | CHIKV-P | HEX-ACAGTGGTTTCGTGTGAGGGCT<br>AC-BHQ1            |  |                                    |
|                     | ZIKV env gene | fwd   | ZIKV-F  | CCGCTGCCCAACACAAG                                |  |                                    |
|                     |               | rev   | ZIKV-R  | CCACTAACGTTCTTTTGCAGACA<br>T                     |  |                                    |
|                     |               | probe | ZIKV-P  | TexasRed-AGCCTACCTTGACAAGCAGTCA<br>GAACTCAA-BHQ2 |  |                                    |
|                     |               |       |         |  |  | CDC Triplex Real-time RT-PCR Assay |

<sup>a</sup> fwd: forward, <sup>b</sup> rev: reverse

Table S2: Criteria for the interpretation of laboratory tests results for Orthoflavivirus diagnosis (ZIKV/DENV) and case classifications

|   | ZIKV  | DENV  | Orthoflavivirus  |
|---|---|---|--|
| <b>Laboratory tests results for <u>probable</u> case</b>  | ZIKV IgM ELISA positive <b>plus</b><br>ZIKV PRNT borderline (case no classified as probable or confirmed for DENV or other Orthoflaviviruses endemic to the region where exposure occurred)   | DENV IgM ELISA positive <b>plus</b><br>DENV PRNT borderline (case no classified as probable or confirmed for ZIKV or other Orthoflaviviruses endemic to the region where exposure occurred)   | - ZIKV and/or DENV IgM ELISA positive <b>plus</b><br>PRNT positive/borderline for ZIKV <b>and</b> DENV (or other Orthoflaviviruses endemic to the region where exposure occurred):<br><b>Probable Recent Orthoflavivirus (PRO)</b> |
| <b>Laboratory tests results for <u>confirmed</u> case</b>   | - Culture of ZIKV from biological sample; <b>OR</b><br>- Detection of ZIKV viral RNA in biological samples [serum, urine, cerebrospinal fluid, placenta, umbilical cord, fetal tissue, or other specimen (e.g., amniotic fluid, semen, saliva)]; <b>OR</b><br>- ZIKV IgM ELISA positive <b>plus</b><br>ZIKV PRNT positive (case not classified as probable or confirmed for DENV or other Orthoflaviviruses endemic to the region where exposure occurred); <b>OR</b><br>- Seroconversion or four-fold antibody titer increase of ZIKV - specific antibodies in paired serum samples <b>plus</b><br>ZIKV PRNT positive (case not classified as probable or confirmed for DENV or other Orthoflaviviruses endemic to the region where exposure occurred) | - Culture of DENV from biological sample; <b>OR</b><br>- Detection of DENV antigen or viral RNA in biological samples; <b>OR</b><br>- DENV IgM ELISA positive <b>plus</b><br>DENV PRNT positive (case no classified as probable or confirmed for ZIKV or other Orthoflaviviruses endemic to the region where exposure occurred); <b>OR</b><br>- Seroconversion or four-fold antibody titer increase of DENV - specific antibodies in paired serum samples <b>plus</b><br>DENV PRNT positive (case no classified as probable or confirmed for ZIKV or other Orthoflaviviruses endemic to the region where exposure occurred) |  |
| <b>Laboratory tests results for <u>excluded</u> infection</b>   | - ZIKV IgM ELISA positive/borderline, not confirmed by PRNT, nor by molecular test<br>- ZIKV PRNT positive/borderline without an increase in the titer in two consecutive samples;<br>- ZIKV IgM ELISA negative/borderline and ZIKV PRNT borderline in a patient with a confirmed, PRNT positive, recent infection by a different Orthoflavivirus   | - DENV IgM ELISA positive/borderline not confirmed by PRNT, nor by molecular test;<br>- DENV PRNT positive/borderline without an increase in the titer in two consecutive samples;<br>- DENV IgM ELISA negative/borderline and DENV PRNT borderline in a patient with a confirmed, PRNT positive, recent infection by a different Orthoflavivirus   |  |
| Laboratory tests results for <b><u>Not Classified</u></b> case (possible past/recent DENV/ZIKV/Orthoflavivirus infection) | - ZIKV IgM ELISA positive/borderline plus ZIKV PRNT bl in late, convalescent samples<br>- ZIKV IgM positive/borderline plus ZIKV PRNT positive/borderline in a patient with a confirmed (molecular test   | - DENV IgM ELISA positive/borderline plus DENV PRNT borderline in late, convalescent samples<br>- DENV IgM positive/borderline plus DENV PRNT positive/borderline in a patient with a confirmed (molecular test   | - ZIKV and/or DENV IgM ELISA negative/borderline, plus PRNT positive/borderline for ZIKV and/or DENV (or other Orthoflaviviruses endemic to the region where exposure occurred)  |

|  |  |  |  |
|--|--|--|--|
|  | positive) recent infection by a<br>different Orthoflavivirus<br>- ZIKV IgM ELISA<br>negative/borderline, plus PRNT<br>positive | positive) recent infection by a<br>different Orthoflavivirus<br>- DENV IgM ELISA<br>negative/borderline, plus PRNT<br>positive |  |
|--|--|--|--|

**Table S3:** DENV and ZIKV confirmed and probable cases' gender and age information. No (%) of cases with this information available, no (%) for each gender and age median [range] and mean ( $\pm$  SD) in years are provided.

|  |                  |                       | <b>Total (%)</b>    |
|--|------------------|-----------------------|---------------------|
| <b>DENV confirmed and probable cases</b> |                  |                       | <b>153 (100,00)</b> |
| <b>Gender</b>                            |                  |                       | 115 (75.16)         |
|  | Male (%)         | 62 (53.91)            |                     |
|  | Female (%)       | 53 (46.09)            |                     |
| <b>Age (in years)</b>                    |                  |                       | 148 (95.48)         |
|  | Median [range]   | 36 [0-82]             |                     |
|  | Mean ( $\pm$ SD) | 36,91 ( $\pm$ 14,72)  |                     |
| <b>ZIKV confirmed cases</b>              |                  |                       | <b>44 (100,00)</b>  |
| <b>Gender</b>                            |                  |                       | 32 (72.72)          |
|  | Male (%)         | 15 (46.88)            |                     |
|  | Female (%)       | 17 (53.13)            |                     |
| <b>Age (in years)</b>                    |                  |                       | 45 (100,00)         |
|  | Median [range]   | 37 [18-68]            |                     |
|  | Mean ( $\pm$ SD) | 39, 07 ( $\pm$ 10.10) |                     |

Table S4: Seroconversion data of confirmed cases for DENV and ZIKV. a. DENV patients that showed seroconversion. b. DENV patients that showed seroconversion. Assay type, first and consecutive result, first and second sample lag time and period for seroconversion are showed.

a.

| DENV    |            |              |               |                              |                               |                              |
|---------|------------|--------------|---------------|------------------------------|-------------------------------|------------------------------|
| Patient | Assay type | First result | Second result | First sample lag time (days) | Second sample lag time (days) | Seroconversion period (days) |
| 1       | PRNT       | neg          | pos           | NA                           | NA                            | 57                           |
| 2       | IgM ELISA  | neg          | pos           | 3                            | 22                            | 19                           |
|         | PRNT       | neg          | b.l.          |                              |                               |                              |
| 3       | PRNT       | b.l.         | pos           | 5                            | 26                            | 21                           |
| 4       | PRNT       | b.l.         | pos           | NA                           | NA                            | NA                           |
| 5       | IgM ELISA  | neg          | pos           | 3                            | 6                             | 3                            |
|         | PRNT       | neg          | b.l.          |                              |                               |                              |
| 6       | IgM ELISA  | pos          | neg           | NA                           | NA                            | 30                           |
| 7       | PRNT       | b.l.         | pos           | 4                            | 25                            | 21                           |
| 8       | IgM ELISA  | b.l.         | pos           | NA                           | NA                            | NA                           |
| 9       | PRNT       | neg          | pos           | 1                            | 12                            | 11                           |
| 10      | PRNT       | neg          | b.l.          | 6                            | 21                            | 15                           |
| 11      | PRNT       | neg          | pos           | 2                            | 15                            | 13                           |
| 12      | PRNT       | neg          | pos           | NA                           | NA                            | 31                           |

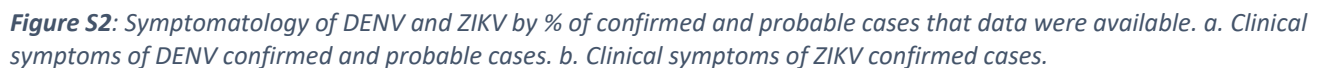
Symptom start is counted as day 0.

b.

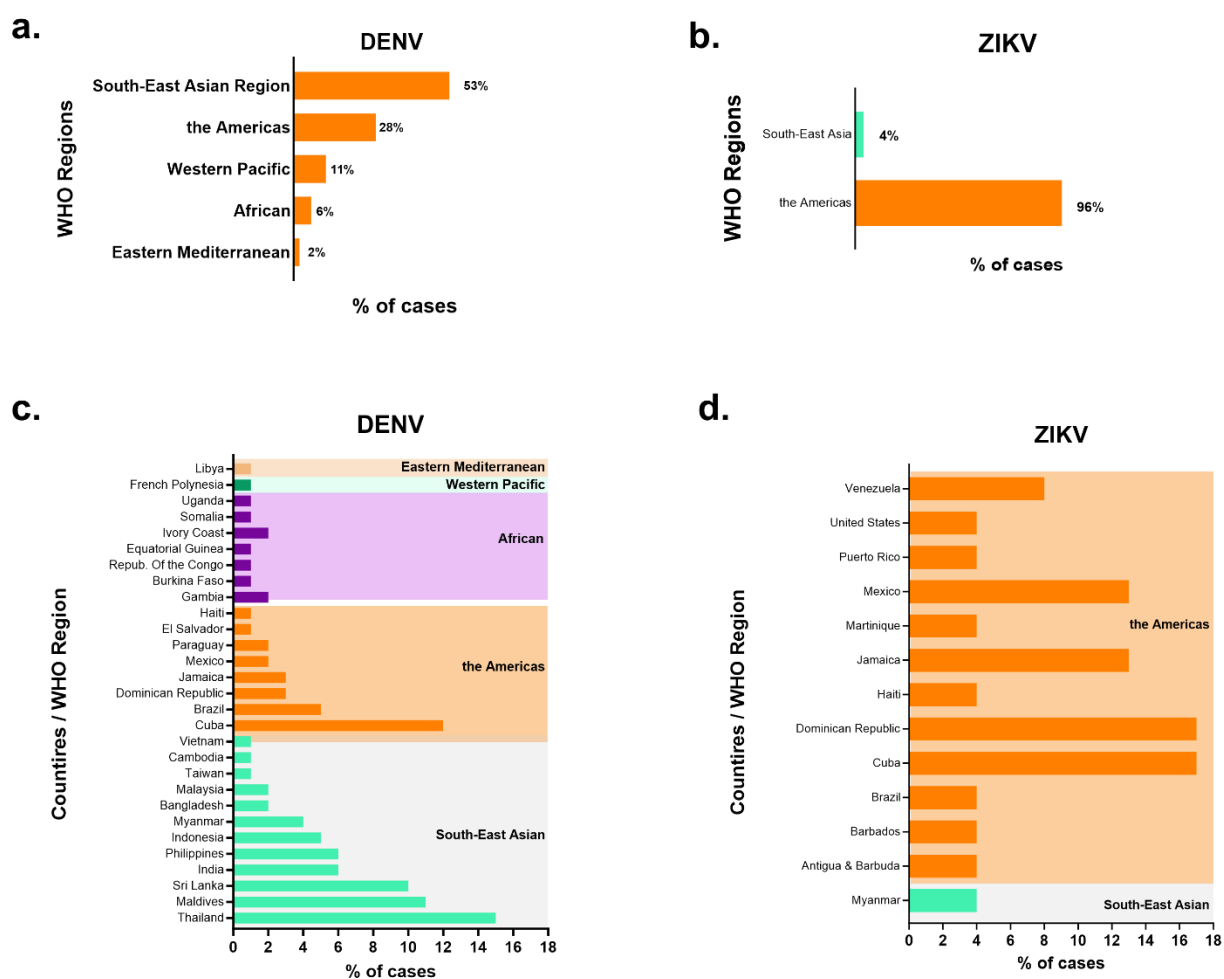
| ZIKV    |            |              |               |                              |                               |                              |
|---------|------------|--------------|---------------|------------------------------|-------------------------------|------------------------------|
| Patient | Assay type | First result | Second result | First sample lag time (days) | Second sample lag time (days) | Seroconversion period (days) |
| 1       | PRNT       | b.l.         | pos           | 7                            | 14                            | 7                            |
| 2       | PRNT       | b.l.         | pos           | NA                           | NA                            | 66                           |
| 3       | PRNT       | b.l.         | Pos           | 19                           | 22                            | 3                            |
| 4       | IgM ELISA  | neg          | pos           | 3                            | 17                            | 13                           |
|         | PRNT       | b.l.         | pos           |                              |                               |                              |

Symptom start is counted as day 0.

Figure S1: Flow chart of the criteria for the interpretation of laboratory tests results for Orthoflavivirus diagnosis (ZIKV/DENV) and case classifications.



**Figure S3**

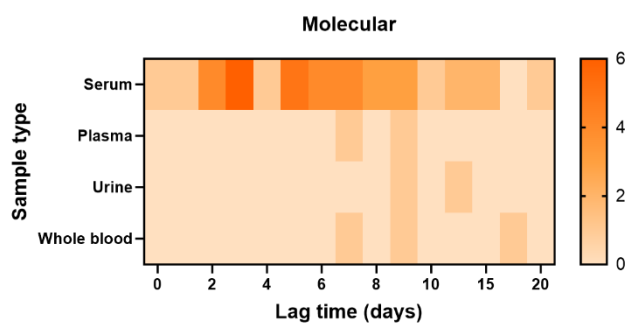


**Figure S3:** Percentages of DENV and ZIKV confirmed and /probable cases by WHO region and by country visited before or during clinical symptoms presentation that data were available. Cases that had visited more than one country are not included in the graphs. a. Percentages of DENV confirmed and probable cases by WHO region. b. Percentages of ZIKV confirmed cases by WHO region. c. Percentages of DENV confirmed and probable cases by country. b. Percentages of ZIKV confirmed cases by country.



Figure S4

**a.**



**b.**

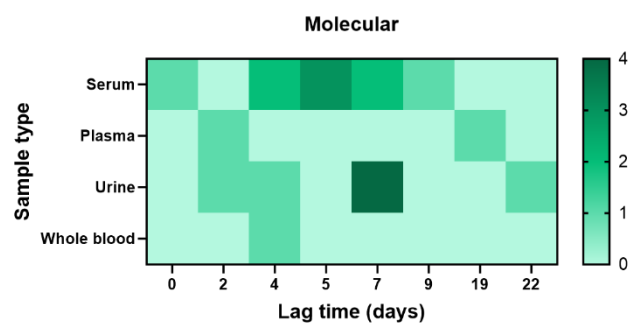


Figure S4: Heat map of sample type, no of samples per type and on lag time days for a. DENV and b. ZIKV that were tested by molecular assay.