# Differential Shedding and Antibody Kinetics of Zika and Chikungunya Viruses, Brazil

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In seroconversion panels obtained from patients from Brazil, diagnostic testing for Zika virus infection was improved by combining multiple antibody isotypes, techniques, and antigens, but sensitivity remained suboptimal. In contrast, chikungunya virus diagnostic testing was unambiguous. Recurrent recent arbovirus infections suggested by serologic data and unspecific symptoms highlight the need for exhaustive virologic testing.

In 2013, Zika virus and chikungunya virus (CHIKV) emerged in Latin America (*1*,*2*). Their overlapping symptoms challenge accurate diagnosis on the basis of clinical manifestations (*3*). Direct Zika virus and CHIKV detection is limited to the acute phase of infection (*4*). Serologic detection of Zika virus–specific antibodies is hampered by low specificity and sensitivity of tests because of immune responses elicited by prior infection with other endemic flaviviruses (e.g., dengue virus [DENV]) (*5*,*6*). In addition, lack of adequate specimens limits studies evaluating the performance of diagnostic tests in tropical areas (*7*,*8*). To evaluate these challenges, we analyzed virus shedding and antibody responses over time in patients in Brazil sampled during the 2016 Zika virus and CHIKV outbreaks.

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## The Study

We prospectively sampled patients in 4 time points up to 90 days post–symptom onset (dpo) (Table 1; Figure 1, panel A; Appendix, https://wwwnc.cdc.gov/EID/article/25/2/18-0166-App1.pdf). The cohort comprised 15 patients with acute Zika virus infection (5 male, 10 female; median age 39.0 years [interquartile range 31.0–44.0 years]) and 18 patients with acute CHIKV infection (10 male, 8 female; median age 39.0 years [interquartile range 31.0–57.3 years]), determined by detection of viral RNA in blood or urine 1–9 dpo (Appendix Figures 1, 2). All Zika virus belonged to the Asian lineage (*2*), and all CHIKV to the East/Central/South African lineage, according to envelope-based typing.

At enrollment, Zika virus patients most frequently reported fever, rash, and arthralgia (80% each), and CHIKV patients most frequently reported arthralgia (100%), fever (89%), and myalgia (89%) (Table 2). No co-infection with Zika virus, CHIKV, or DENV was detected by real-time reverse transcription PCR (rRT-PCR). However, serologic analyses found that 4 (27%) Zika virus–infected patients also had CHIKV IgM at enrollment, and 1 (7%) had DENV IgM (Appendix Table 1, Figure 3). Similarly, 3 (17%) CHIKV-infected patients had Zika virus IgM, and 4 (22%) CHIKV-infected patients had DENV IgM at enrollment (Appendix Figure 4). We cannot exclude the possibility of cross-reactivity between Zika virus–specific and DENV-specific antibodies because 2 CHIKV patients simultaneously showed Zika virus and DENV IgM in an envelope-based ELISA (Appendix Table 2). Seventy-nine percent of Zika virus and 83% of CHIKV patients showed serologic evidence for past DENV infection at enrollment (Appendix Figures 1, 2). Thus, recent infections with heterologous arboviruses might bias attributing infection-specific symptoms for Zika virus and CHIKV.

Consistent with previous studies (*4*,*9*), Zika virus loads in serum and urine were low up to 9 dpo (≈$10^4$ RNA copies/mL) (Figure 1, panel B), whereas CHIKV loads were ≈100-fold higher (≈$10^6$ RNA copies/mL) (Figure 1, panel C). However, unlike with Zika virus, CHIKV loads decreased significantly ($p<0.001$ by *t* test) from 5 dpo onward, and viral loads in urine were consistently low (Figure 1, panels D, E).

Next, to assess the antibody kinetics of Zika virus and CHIKV, we measured antibody responses over time

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**Table 1.** Sampling details for retrospective study of differential shedding and antibody kinetics of Zika virus and CHIKV, Brazil, 2016*

| Sample no. | Virus detected | Days from symptom onset to sampling | Collection date of acute-phase samples |
|---|---|---|---|
| DQ005 | Zika virus | 2 | Mar 14 |
| DQ028 | Zika virus | 1 | Mar 21 |
| DQ042 | Zika virus | 3 | Mar 23 |
| DQ47 | Zika virus | 2 | Mar 28 |
| DQ049 | Zika virus | 1 | Mar 28 |
| DQ058 | Zika virus | 4 | Mar 30 |
| DQ60 | Zika virus | 2 | Mar 30 |
| DQ62 | Zika virus | 3 | Mar 30 |
| DQ75 | Zika virus | 3 | Apr 4 |
| DQ77 | Zika virus | 5 | Apr 5 |
| DQ108 | Zika virus | 2 | Apr 13 |
| DQ116 | Zika virus | 2 | Apr 14 |
| DQ125 | Zika virus | 3 | Apr 18 |
| DQ131 | Zika virus | 5 | Apr 18 |
| DQ246 | Zika virus | 5 | Jun 24 |
| DQ030 | CHIKV | 3 | Mar 21 |
| DQ045 | CHIKV | 5 | Mar 24 |
| DQ054 | CHIKV | 2 | Mar 30 |
| DQ056 | CHIKV | 2 | Mar 30 |
| DQ057 | CHIKV | 3 | Mar 30 |
| DQ061 | CHIKV | 2 | Mar 30 |
| DQ071 | CHIKV | 4 | Apr 4 |
| DQ074 | CHIKV | 1 | Apr 4 |
| DQ079 | CHIKV | 3 | Apr 5 |
| DQ083 | CHIKV | 3 | Apr 6 |
| DQ085 | CHIKV | 4 | Apr 7 |
| DQ097 | CHIKV | 3 | Apr 11 |
| DQ113 | CHIKV | 5 | Apr 13 |
| DQ144 | CHIKV | 4 | Apr 25 |
| DQ170 | CHIKV | 2 | May 3 |
| DQ195 | CHIKV | 2 | May 11 |
| DQ210 | CHIKV | 2 | May 16 |
| DQ220 | CHIKV | 4 | May 17 |

*CHIKV, chikungunya virus.

by commercial and in-house serologic tests. In a widely used nonstructural (NS) protein 1 antigen-based ELISA, Zika virus IgM seroconversion was low (33% [5/15]), whereas CHIKV IgM seroconversion was 100% using an envelope-based ELISA ($p<0.0001$ by Fisher exact test) (Figure 2, panel A; Appendix Tables 1, 2). Use of an in-house envelope-based ELISA increased the Zika virus IgM detection rate to 50% (7/14), and use of a commercially available μ-capture ELISA increased it to 43% (6/14) (Figure 2, panel A). Despite differential sensitivity, concordant results from different assays suggest comparable specificity of IgM detection (Appendix Table 1). The use of NS1-based IgA as a marker of acute infection increased the detection rate to 53% (8/15) over that of the NS1-based IgM ELISA. All IgM-positive patients also showed IgA, which increased during acute and subacute phases of infection and decreased during convalescence (Figure 2, panel B; Appendix Figure 3). This finding supports the usability of IgA-based serologic methods as an alternative or additional marker to IgM-based methods to detect acute Zika virus infection. The detection rate increased 2-fold when we used NS1-based IgA from when we used NS1-based IgM 5–9 dpo, suggesting that IgA could be used at later stages of infection (Appendix Figures 1, 5). Our findings indicate that serologic detection of acute Zika virus infection can be improved ≈2-fold by use of different antibody classes and antigens but remains poorly sensitive in flavivirus-endemic areas.

All Zika virus–infected patients showed IgG responses across the 4 time points in ≥1 assay (Figure 2, panels C, D). Plaque reduction neutralization tests (PRNTs) were negative for 2 of 14 rRT-PCR–confirmed Zika virus cases detected by NS1-based IgG ELISA. Without rRT-PCR confirmation, these cases would have been classified false positive (Appendix Table 1). This observation might be explained by differential sensitivity of PRNT and ELISA (*10*) or false-positive results of the Zika virus NS1-based ELISA in secondary flavivirus infections (*6*). Similarly, the antibody kinetics of Zika virus NS1-based IgG, envelope-based IgG, and PRNT suggested either relatively early IgG seroconversion or cross-reactivity during acute stages of infection resulting from unspecific immune responses against other flaviviruses (*11*) (Figure 2, panel D). In contrast, CHIKV IgG seroconversion occurred at later stages (Figure 2, panel D; Appendix Figure 5), possibly associated with strong and long-lasting CHIKV-specific IgM responses (Appendix Figure 4).

## Conclusions

We provide pivotal data on Zika virus and CHIKV diagnostic challenges in a Latin American setting. Limitations of our study include the relatively small number of patients, sampling at heterogeneous dpo and heterogeneous numbers of samples per dpo, and lack of acutely DENV-infected patients to assess test specificity. The strengths of our study include rRT-PCR–confirmed infections, waiving the need to define serologic assays prone to cross-reactivity as standards, sampling during Zika virus and CHIKV outbreaks (*1*,*2*), sequential sampling of patients up to 90 dpo, use of multiple antigens and immunoglobulin classes, and the combination of molecular and serologic testing methods.

Our data suggest reliable diagnostic testing for acute CHIKV infections by IgM detection from 5 dpo onward. This finding might enable waiving labor-intense and costly molecular protocols in many patients, minimizing costs for public health systems and cohort studies investigating arbovirus pathogenesis. However, reliability of CHIKV serologic diagnostic tests must be reevaluated for co-circulating genotypes (*12*) and for the antigenically related Mayaro virus (*13*) if it emerges in Latin America.

The difficulties of adequately diagnosing Zika virus infections in areas to which it is endemic have major

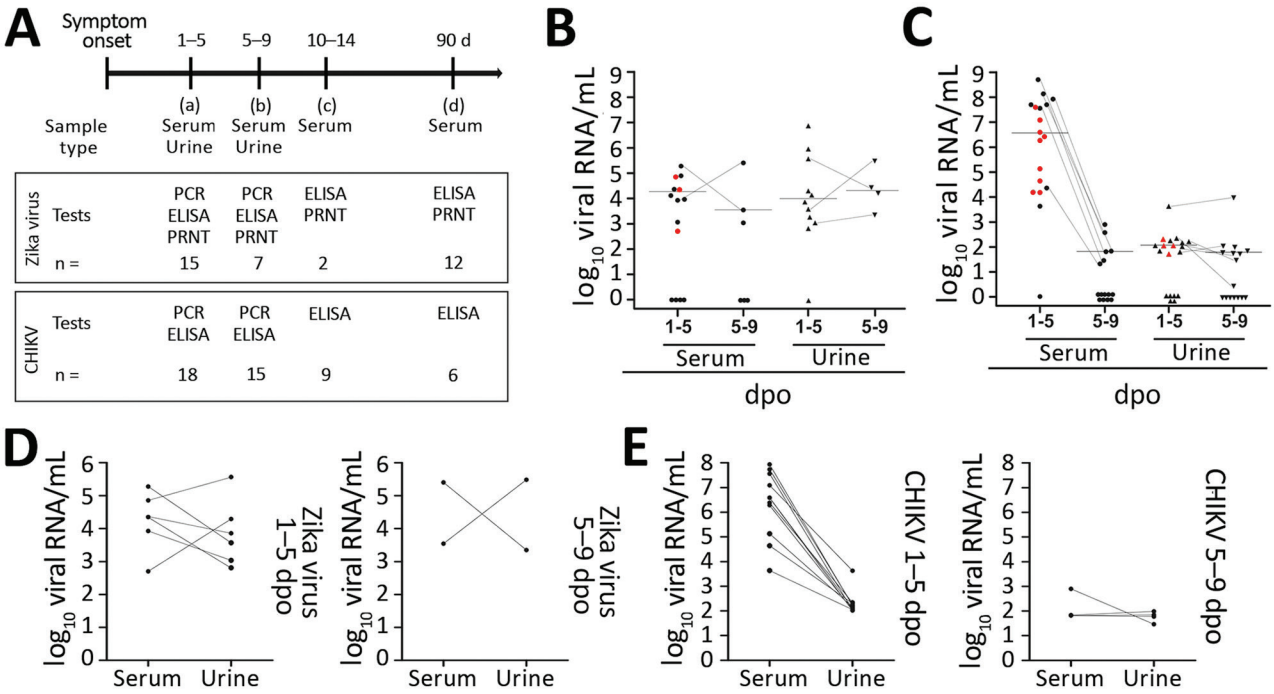


**Figure 1.** Overview of diagnostic testing and shedding dynamics for Zika virus and CHIKV among patients in Brazil, 2016. A) Timeline of sampling and number of samples for each test. B, C) Zika virus (B) and CHIKV (C) loads in different body fluids at 1–5 and 5–9 dpo. Black dots indicate single samples. Red dots indicate samples taken 1–5 dpo that were negative 5–9 dpo. Dotted lines indicate paired samples that were positive at both time points. Bold line indicates the median. D, E) Viral loads of Zika virus (D) and CHIKV (E) in paired urine and serum samples from individual patients, 1–5 and 5–9 dpo. Data were analyzed using GraphPad Prism 5 (GraphPad Software, Inc., https://www.graphpad.com). CHIKV, chikungunya virus; dpo, days post–symptom onset; PRNT, plaque reduction neutralization test.

implications for public health. Reliable testing for flaviviruses in such areas will be key for epidemiologic studies on Zika virus and assessments of the safety of flavivirus vaccination programs, as illustrated by more severe dengue infections in DENV-seronegative individuals who received a live attenuated dengue vaccine (*14*).

For pregnant women and couples intending pregnancy, accurate diagnosis of acute or past Zika virus infection is crucial. The steep increase in requests for abortion in Latin America illustrates the effect of the Zika virus outbreak on reproductive medicine (*15*).

**Table 2.** Symptoms of Zika virus and CHIKV reported by patients at enrollment 1–5 days after symptom onset, Brazil, 2016*

| Symptom | Zika virus, no. (%), n = 15 | CHIKV, no. (%), n = 18 |
|---|---|---|
| Rash | 12 (80) | 9 (50) |
| Fever | 12 (80) | 16 (89) |
| Arthralgia | 12 (80) | 18 (100) |
| Myalgia | 9 (60) | 16 (89) |
| Cephalea | 8 (53) | 12 (67) |
| Retro-orbital pain | 5 (33) | 8 (44) |
| Edema | 4 (27) | 3 (17) |
| Nausea, vomiting | 3 (20) | 6 (33) |
| Conjunctivitis | 2 (13) | 5 (28) |

*CHIKV, chikungunya virus.

Our results highlight that definite exclusion of acute Zika virus infections is challenging in a considerable proportion of patients. However, although limited by a small number of samples, our data highlight the attainability of more accurate Zika virus diagnostic testing by combining molecular and serologic tests using different antibody classes, antigens, and methods and by monitoring an increase of IgG titers in follow-up serum samples. Our data will help clinicians and health authorities build reliable diagnostic algorithms for Zika virus and CHIKV and highlight that exhaustive testing of arboviral infections is required for attributing frequencies of infection-specific symptoms.

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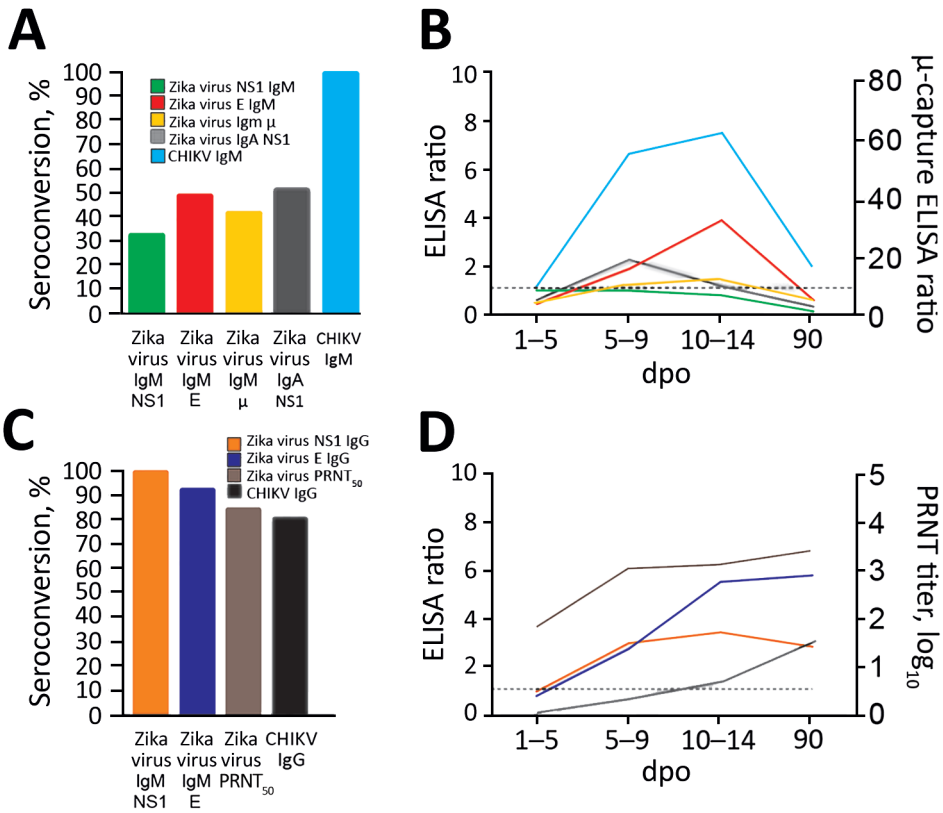


**Figure 2.** Zika virus and CHIKV antibody dynamics among samples from patients in Brazil, 2016. A) Percentage seroconversion for markers of acute infection with Zika virus and CHIKV (IgM NS1–based Zika virus ELISA, IgM envelope-based Zika virus ELISA, IgM μ-capture Zika virus ELISA, IgA NS1-based Zika virus ELISA, IgM CHIKV ELISA) at any time point. B) Median ELISA ratios for Zika virus and CHIKV IgM and IgA over time. C) Percentage seroconversion for markers of convalescence after Zika virus and CHIKV infection (IgG NS1-based Zika virus ELISA and IgG envelope-based Zika virus ELISA, Zika virus $PRNT_{50}$, IgG CHIKV ELISA) at any time point. D) Median ELISA ratios for Zika virus and CHIKV IgG over time. Numbers of specimens per time point are shown in Figure 1. Dashed lines indicate signal-to-cutoff ratios of ≥1.1 considered positive for all ELISAs except μ-capture ELISA, for which the dashed line indicates a signal-to-cutoff ratio of ≥10, considered positive by the manufacturer. See Appendix Figure 5 (https://wwwnc.cdc.gov/EID/article/25/2/18-0166-App1.pdf) for the percentage de novo seroconversion of Zika virus and CHIKV in different assays per time point. CHIKV, chikungunya virus; dpo, days post–symptom onset; E, envelope; NS, nonstructural protein; PRNT, plaque reduction neutralization test.

## About the Author

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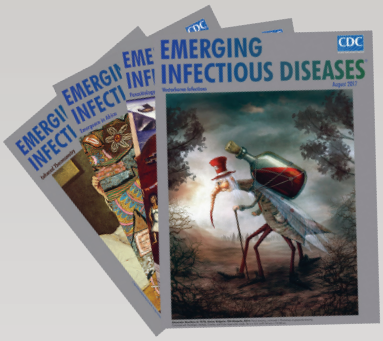


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# Differential Shedding and Antibody Kinetics of Zika Virus and Chikungunya Virus, Brazil

## Appendix

**Appendix Table 1.** Serologic data for 14 Zika virus patients, Brazil, 2016*

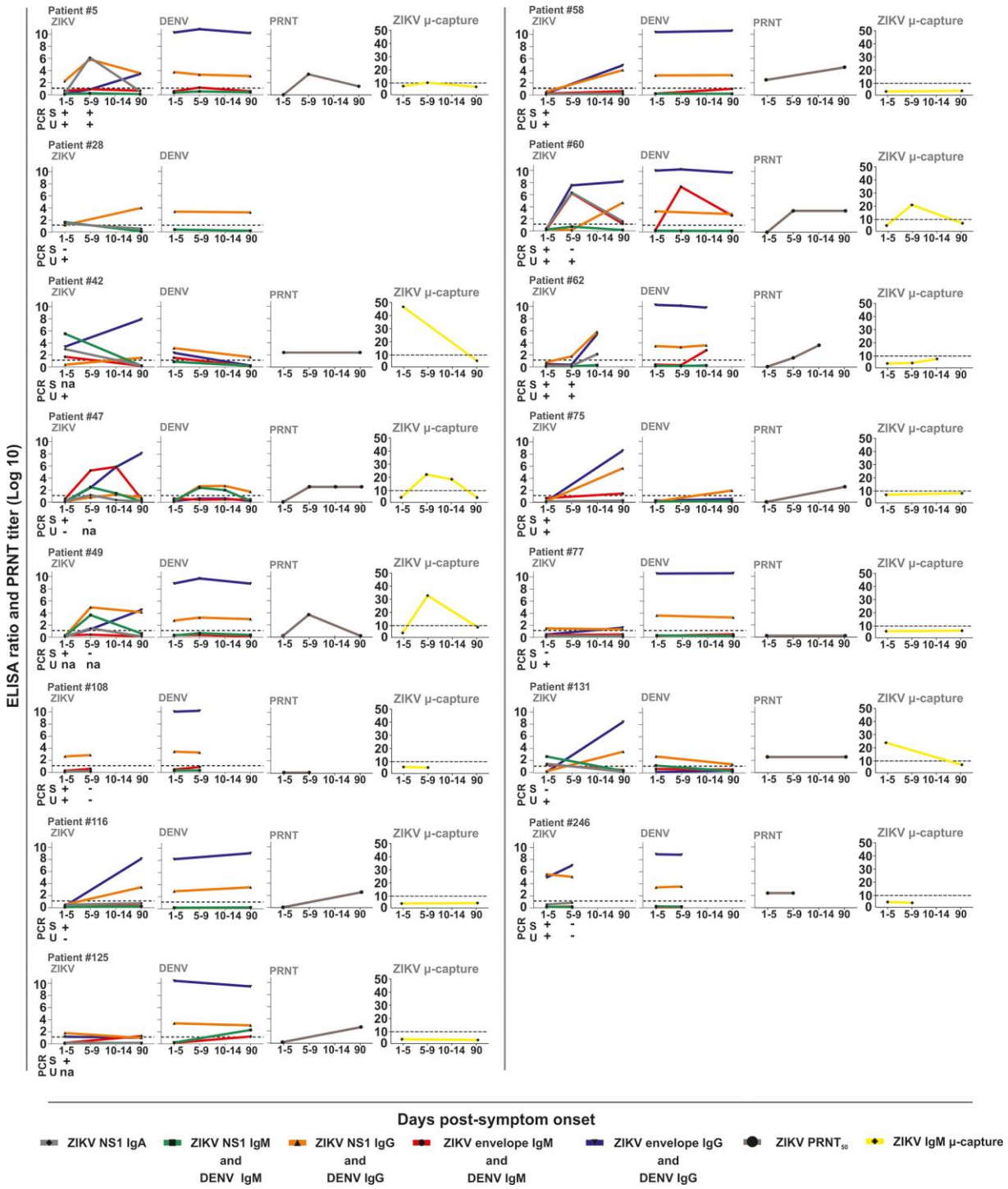
| | | Zika virus | | | | | | | CHIVK | | DENV | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Age, y | **IgA NS1** | **IgM NS1** | **IgM E** | **IgM μ** | **IgG NS1** | **IgG E** | **PRNT 50** | **IgM RE** | **IgG RE** | **IgA FV** | **IgM FV** | **IgM E** | **IgG FV** | **IgG E** |
| DQ005 | 32 | + | – | – | + | + | + | + | – | – | – | – | + | + | + |
| DQ028 | 44 | + | + | NA | NA | + | NA | NA | – | – | – | – | NA | + | NA |
| DQ042 | 35 | + | + | + | + | + | + | + | + | – | – | – | + | + | – |
| DQ47 | 36 | + | + | + | + | + | + | + | – | – | – | + | – | + | – |
| DQ049 | 40 | + | + | – | + | + | + | + | – | – | – | – | – | + | + |
| DQ058 | 39 | – | – | – | – | + | + | + | – | – | – | – | – | + | + |
| DQ60 | 60 | + | – | + | + | + | + | + | – | – | – | – | + | + | + |
| DQ62 | 44 | + | – | + | – | + | + | + | – | – | – | – | + | + | + |
| DQ75 | 30 | – | – | + | – | + | + | + | – | – | – | – | – | + | – |
| DQ77 | 28 | – | – | – | – | + | + | – | + | + | + | – | – | + | + |
| DQ108 | 21 | – | – | – | – | + | – | – | – | – | – | – | – | + | + |
| DQ116 | 46 | – | – | – | – | + | + | + | – | – | – | – | – | + | + |
| DQ125 | 41 | – | – | + | – | + | + | + | + | + | + | + | + | + | + |
| DQ131 | 31 | + | + | + | + | + | + | + | – | – | – | + | – | + | – |
| DQ246 | NA | – | – | – | – | + | + | + | + | + | + | – | – | + | + |
| Total | | 8 | 5 | 7 | 6 | 15 | 13 | 12 | 4 | 3 | 3 | 3 | 5 | 15 | 10 |

*ELISA or PRNT results at any time point of sampling. CHIKV, chikungunya virus; DENV, dengue virus; E, envelope; FV, full virus; NS, nonstructural protein; NA, samples not available; PRNT, plaque-reduction neutralization test; RE, recombinant; +, positive; –, negative.

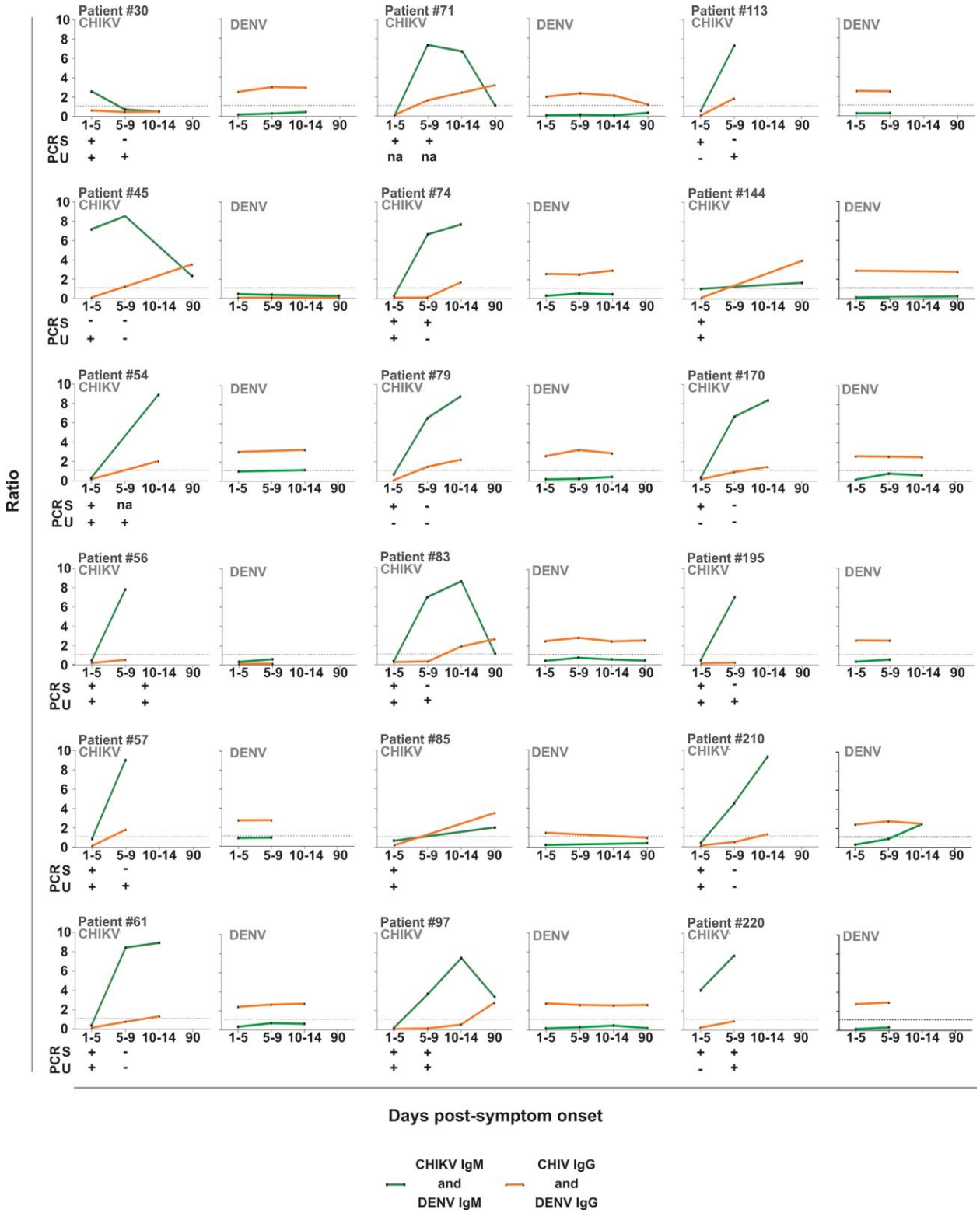
**Appendix Table 2.** Serologic data for 18 CHIKV patients, Brazil, 2016*

| | | Zika virus | | | | | | CHIKV | | DENV | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Age, y | **IgA NS1** | **IgM NS1** | **IgM E** | **IgM μ** | **IgG NS1** | **IgG E** | **IgM RE** | **IgG RE** | **IgM FV** | **IgM E** | **IgG FV** | **IgG E** |
| DQ030 | 45 | + | – | – | – | + | + | + | – | – | – | + | + |
| DQ045 | 27 | – | – | – | – | – | – | + | + | – | – | – | – |
| DQ054 | 57 | – | – | – | – | + | + | + | + | + | – | + | + |
| DQ056 | 41 | – | – | + | – | – | – | + | – | – | + | – | – |
| DQ057 | 38 | – | – | – | – | + | – | + | + | – | – | + | + |
| DQ061 | 35 | – | – | – | – | + | – | + | + | – | + | + | + |
| DQ071 | 69 | – | – | – | – | – | – | + | + | – | – | + | + |
| DQ074 | 33 | – | – | – | – | + | + | + | + | – | – | + | + |
| DQ079 | 33 | – | – | – | – | + | + | + | + | – | – | + | + |
| DQ083 | 58 | + | – | – | – | + | + | + | + | – | + | + | + |
| DQ085 | 40 | – | – | – | – | + | + | + | + | + | – | + | – |
| DQ097 | 29 | – | – | – | – | – | – | + | + | – | – | + | + |
| DQ113 | 42 | – | – | – | – | + | + | + | + | – | – | + | + |
| DQ144 | 31 | – | – | – | – | + | + | + | + | – | – | + | + |
| DQ170 | 24 | – | – | + | – | + | + | + | + | – | + | + | + |
| DQ195 | 31 | – | – | – | – | – | – | + | – | – | – | + | + |
| DQ210 | 75 | – | – | – | – | – | – | + | + | – | – | + | + |
| DQ220 | 85 | – | – | – | – | + | + | + | – | – | – | + | + |
| Total positive | | 2 | 0 | 2 | 0 | 12 | 10 | 18 | 15 | 2 | 4 | 16 | 15 |

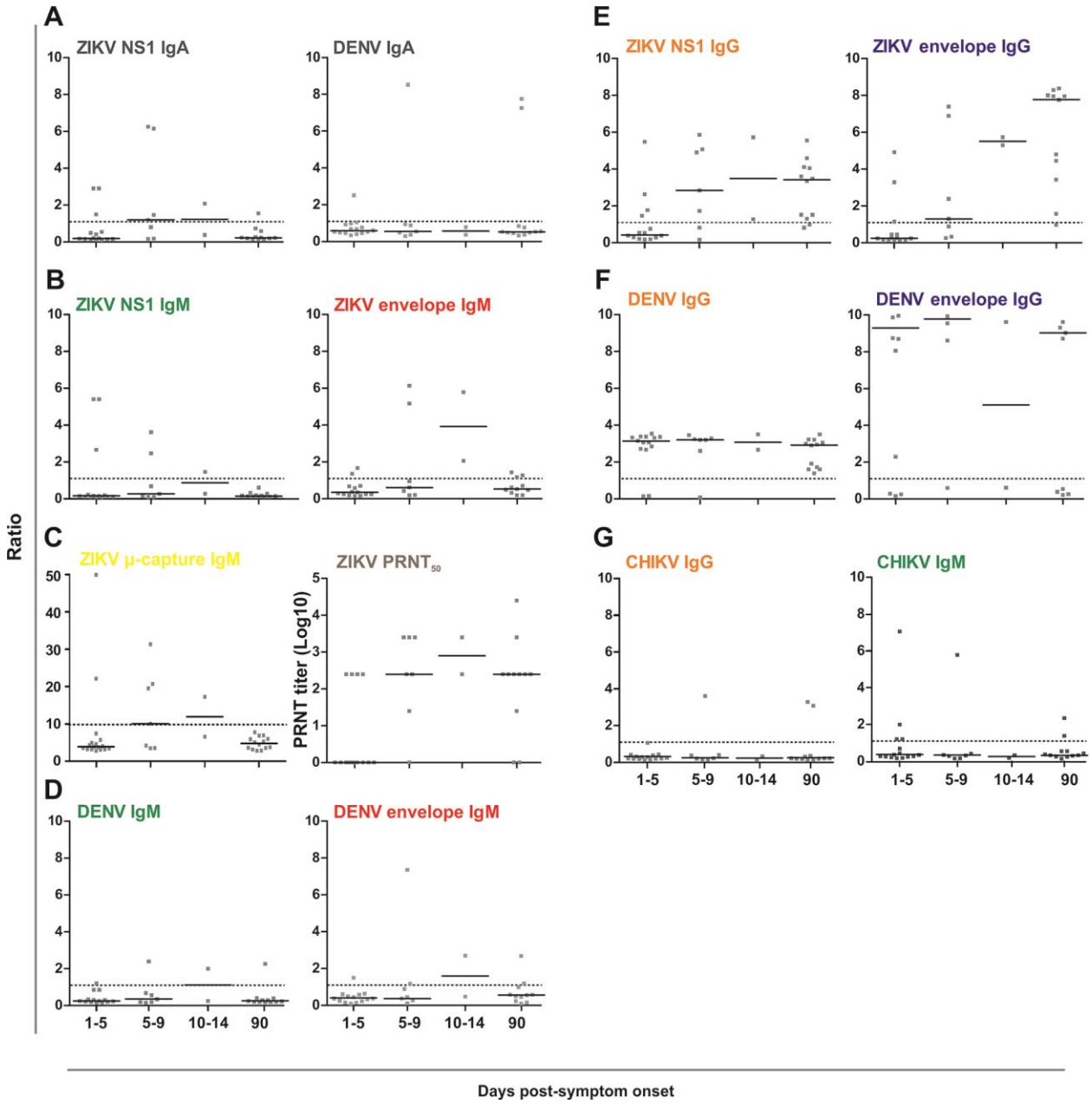
*ELISA results at any time point of sampling. CHIKV, chikungunya virus; DENV, dengue virus; E, envelope; FV, full virus; NS, nonstructural protein; RE, recombinant; +, positive; –, negative.



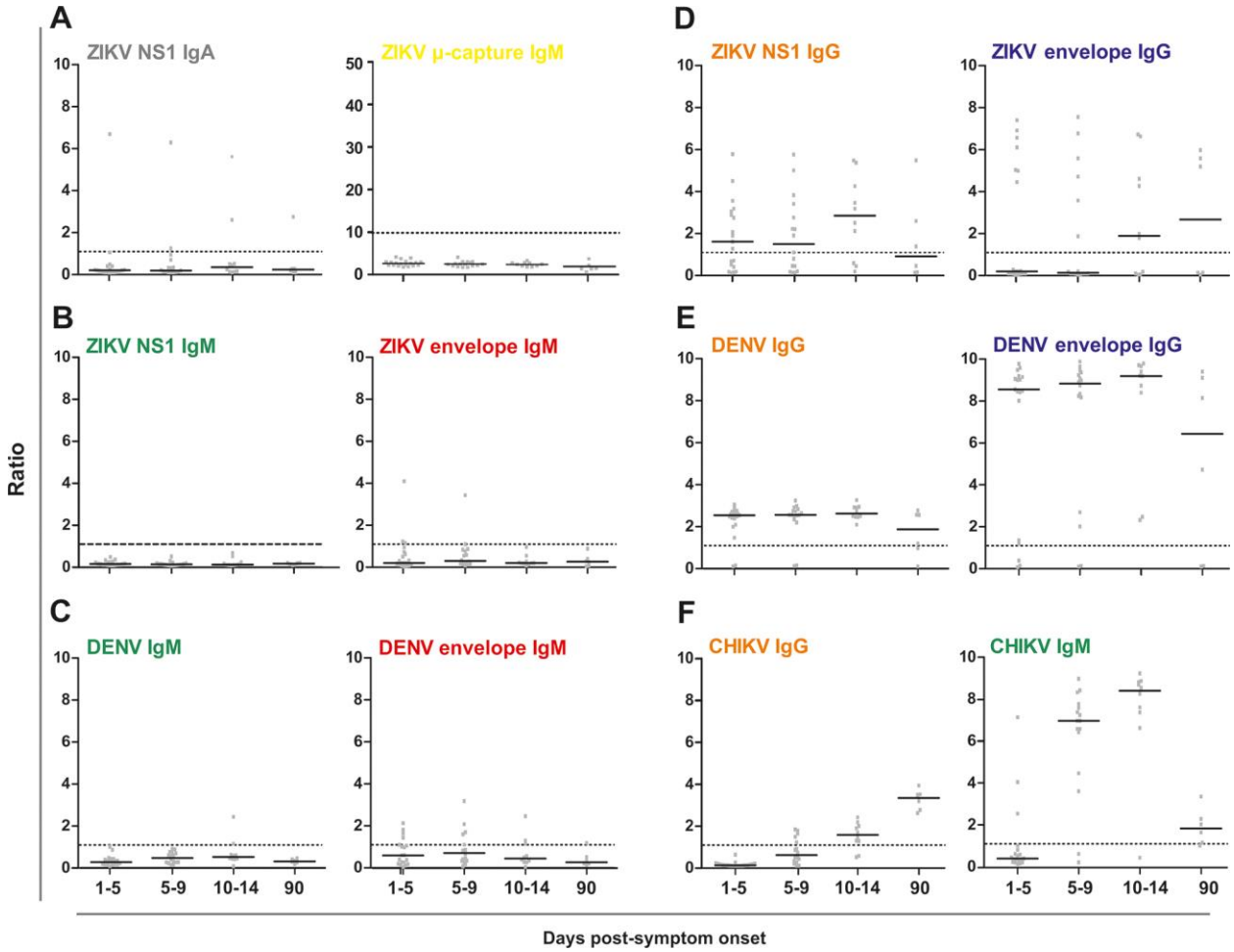

**Appendix Figure 1.** Antibody kinetics and real-time reverse transcription PCR results for ZIKV and DENV of 14 Zika patients. Below each panel, PCR results for serum and urine are shown. DENV, dengue virus; NA, sample not available; NS, nonstructural protein; PRNT, plaque-reduction neutralization test; ZIKV, Zika virus.



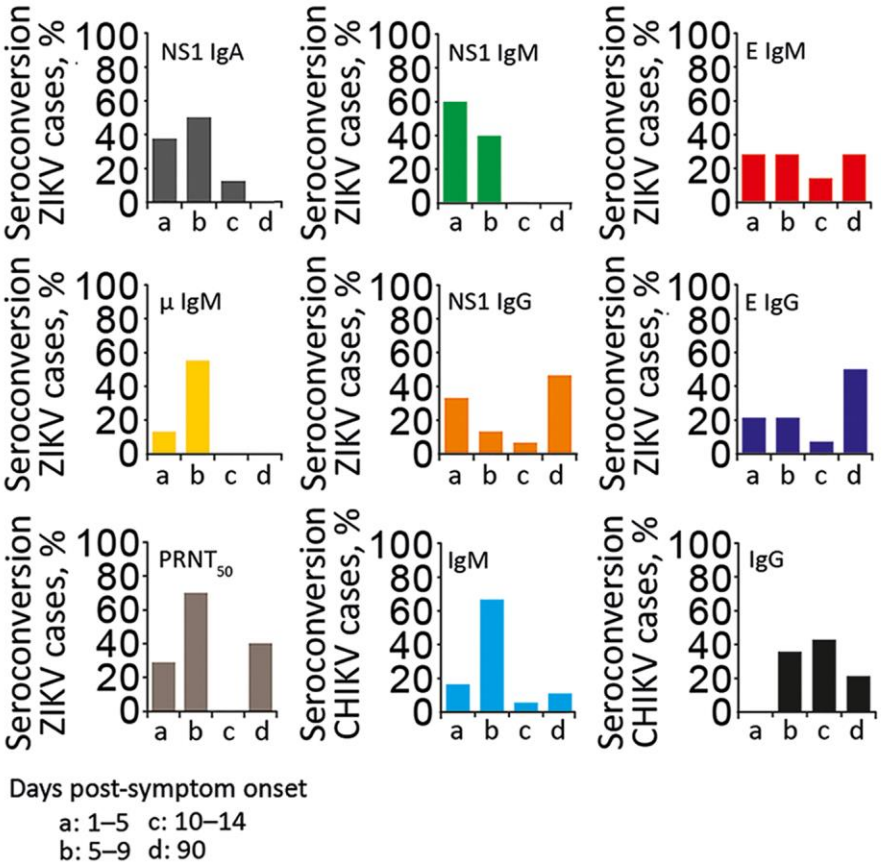

**Appendix Figure 2.** Antibody kinetics and real-time reverse transcription PCR results for CHIKV and DENV of 18 chikungunya patients, Brazil, 2016. Below each panel, PCR results for serum (S) and urine (U) are shown. CHIKV, chikungunya virus; DENV, dengue virus.




**Appendix Figure 3.** ELISA ratios and plaque-reduction neutralization test titers of 14 Zika virus–positive patients, Brazil, 2016. Lines show the median. Dashed lines indicate signal-to-cutoff ratios of ≥1.1 considered positive. For the μ-capture ELISA, the dashed line indicates a signal-to-cutoff ratio of ≥10. CHIKV, chikungunya virus; DENV, dengue virus; NS, nonstructural protein; PRNT, plaque-reduction neutralization test; ZIKV, Zika virus




**Appendix Figure 4.** ELISA ratios for 18 CHIKV-positive patients, Brazil, 2016. Dashed lines indicate signal-to-cutoff ratios of ≥1.1 considered positive. For the μ-capture ELISA, dashed line indicates a signal-to-cutoff ratio of ≥10. CHIKV, chikungunya virus; DENV, dengue virus; NS, nonstructural protein; ZIKV, Zika virus.




**Appendix Figure 5.** Percentage de novo seroconversion of Zika virus and CHIKV in different assays per time point. Total numbers of patients that seroconverted for Zika virus per assay, antigen, and antibody were as follows: NS1 IgA (n = 8), NS1 IgM (n = 5), envelope IgM (n = 7), NS1 IgG (n = 15), envelope IgG (n = 14) μ-capture IgM (n = 6), and PRNT50 (n = 12). Total numbers of specimens that seroconverted for CHIKV were as follows: IgM (n = 18), IgG (n = 14). NS, nonstructural protein; PRNT, plaque-reduction neutralization test.