

**TITLE: PERSISTENCE OF ZIKA VIRUS IN SEMEN 93 DAYS AFTER THE ONSET OF SYMPTOMS**

**TÍTULO: PERSISTENCIA DE VIRUS ZIKA EN SEMEN 93 DÍAS TRAS EL COMIENZO DE LOS SÍNTOMAS**

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**Abstract**

**Introduction:** Zika virus is transmitted mainly through the bites of infected Aedes mosquitoes, although mother-to-child and sexual transmission have also been described. The presence of zika virus in semen after infection seems to be not uncommon, but the duration of viral persistence is not well determined.

**Methods:** We used molecular, serological and cell culture methods for the diagnosis and follow up of a case of zika virus infection imported from Venezuela. Serial samples of serum, urine and semen were analyzed to investigate zika virus persistence.

**Results:** zika virus was detected in semen samples up to 93 days after the onset of symptoms.

**Conclusions:** Our results confirm persistence of zika virus in semen samples for long periods after infection

## Resumen

**Introducción:** El virus zika se transmite fundamentalmente por la picadura de mosquitos Aedes infectados, aunque también es posible la transmisión de madre a hijo y la transmisión sexual. La presencia del virus Zika en semen tras la infección parece ser algo relativamente frecuente, pero la duración de la persistencia viral no es bien conocida.

**Métodos:** Mediante técnicas moleculares, serológicas y cultivo celular se diagnóstico un caso de zika importado de Venezuela y se tomaron muestra seriadas de suero, orina y semen para investigar la persistencia del virus

**Resultados:** El virus zika fue detectado en muestras de semen recogidas 93 días después del inicio de los síntomas

**Conclusiones:** Nuestros resultados confirman la persistencia del virus zika en semen por períodos prolongados de tiempo después de la infección

**Key words:** zika virus; semen; persistence

**Palabras clave:** virus zika; semen; persistencia

## Case report

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3 In January 2016 a man in his 30s, previously diagnosed with prostatitis in December  
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6 2015 and in treatment with ciprofloxacin, presented to the Tropical Medicine  
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8 Department at Hospital Clinic (Barcelona) two days after his return from a 15-day  
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10 holiday trip to Venezuela. During his stay he reported self-limited hematospermia and  
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12 ceftibuten was added by a local urologist to his previous ciprofloxacin regime. In the  
13  
14 trip back to Europe he started with malaise, fever ( $\geq 39^{\circ}\text{C}$ ), myalgias, arthralgias and  
15  
16 retro-orbital pain. One day after, he developed a total itchy body macular rash,  
17  
18 followed by conjunctivitis. He had not been vaccinated for this trip. He had received  
19  
20 yellow fever vaccination in the past and referred dengue fever infection during his  
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22 childhood. Medical examination revealed bilateral enlarged latero cervical lymph  
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24 nodes, non purulent conjunctivitis and an intense itchy maculopapular rash diffused on  
25  
26 the face, trunk, arms and legs. He had mild transaminitis (alanine aminotransferase  
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28 195 UI/L, aspartate aminotransferase 79 UI/L) and elevated C-reactive protein (1.69  
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30 mg/dl). A complete blood count, blood levels of electrolytes, as well as coagulation and  
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32 renal-function tests were normal. Chest radiograph results were unremarkable. The  
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34 fever and the cutaneous rash resolved in 3 days while malaise, weakness, and myalgia  
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36 lasted for 1 week after presentation.  
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## Laboratory investigations

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55 Diagnosis of zika virus (ZIKV) infection was established by real time reverse-  
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57 transcription polymerase chain reaction (RT-PCR) using a commercial kit (**RealStar Zika**  
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59 **Virus RT-PCR Kit 1.0, Altona Diagnostics, Hamburg, Germany**) in a serum sample  
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1 collected three days post onset of illness (dpi). Dengue and chikungunya RNA were not  
2 detected by specific real-time RT-PCR assays and malaria was ruled out by negative  
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4 blood thick and thin smears. **The presence of ZIKV was confirmed by Sanger**  
5  
6 **sequencing analysis of a 270 bp amplicon (from a generic flavivirus RT-PCR assay<sup>1</sup>) of**  
7  
8 **the NS5 gene.** The patient agreed to provide follow up samples to investigate the  
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10 presence of ZIKV in semen, serum and urine samples. A summary of the samples  
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12 collected and the results obtained is shown in Table 1. No ZIKV RNA was detected in  
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14 follow up serum or urine samples. The first semen sample was obtained at 30 dpi and  
15  
16 tested positive by ZIKV real time RT-PCR. Two more semen samples obtained at 51 and  
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18 93 dpi also contained detectable levels of ZIKV RNA. A fourth semen sample collected  
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20 201 dpi tested negative for ZIKV. **For cell culture, semen samples were kept frozen**  
21  
22 **until inoculated onto Vero cells and monitored for virus growth by visual inspection**  
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24 **of the cytopathic effect and by RT-PCR of culture supernatants, but the virus could**  
25  
26 **not be isolated.** Serological tests were performed for dengue (Panbio ELISA, Alere,  
27  
28 Australia), chikungunya and zika (Immunofluorescence, Euroimmun, Germany). The  
29  
30 patient tested positive for IgG against dengue upon first presentation at the Hospital,  
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32 in agreement with the dengue virus infection he reported to have suffered during his  
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34 childhood. This first sample tested positive for IgG against zika by immunofluorescence  
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36 but was negative by Anti-Zika Virus ELISA (Euroimmun, Germany), in concordance with  
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38 the higher specificity reported for the ELISA test<sup>2</sup>. IgM against zika was positive one  
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40 month after onset of symptoms and was undetectable 2 months later.  
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## Discussion

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3 ZIKV is, to date, the only flavivirus transmitted through sexual contact, and this mode  
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5 of transmission might be more frequent than previously thought. So far, male to male  
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8 <sup>3</sup>, male to female <sup>4,5</sup> and female to male sexual transmission <sup>6</sup> have been documented  
9  
10 and up to 11 countries have reported ZIKV sexual transmission. While most cases have  
11  
12 been originated from men with previous history of ZIKV symptoms, the virus can also  
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14 be spread through sexual contact before the symptoms develop and even from  
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16 asymptomatic ZIKV infections <sup>7</sup>. ZIKV sexual transmission is of public health concern,  
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18 particularly for pregnant women given the possibility of ZIKV induced fetal  
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20 abnormalities <sup>8</sup>.  
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28 The duration of ZIKV persistence in semen is yet poorly understood. Reports of  
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30 travelers that acquired ZIKV during the present American epidemic showed viral  
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32 persistence in semen for 47, 62, 76 and 80 days. Recently, a case of Zika Virus  
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34 persistence for 6 months in semen has been described in a traveler returning from  
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36 Haiti to Italy<sup>9</sup>. In our case, ZIKV infection imported from Venezuela was confirmed  
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38 during the viremic period 3 days after the onset of symptoms. Results of the follow up  
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40 samples suggest that viral persistence was restricted to semen and longer shedding in  
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42 urine was not demonstrated. Although it is possible to isolate ZIKV from semen, our  
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44 attempts were not successful. Failure to isolate ZIKV from semen samples has been  
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46 reported in other studies<sup>9</sup>, with even higher viral loads (lower Ct) than in our case. The  
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48 RT-PCR Cycle Threshold (Ct) values obtained in the three positive semen samples  
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50 remained relatively stable until the last positive sample was tested as long as 93 days  
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52 after the onset of symptoms. Our results confirm longer periods for ZIKV presence in  
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1 semen and represent, to our knowledge, the first reported follow up of zika virus in  
2 semen in Spain. Unfortunately, samples between the third and the sixth month after  
3 symptomatic illness were not available for testing. We can only suggest that  
4 somewhere between 93 and 201 dpi ZIKV RNA declined to undetectable levels. Current  
5 recommendations include a precautionary period of safer sex/abstinence for 6 months  
6 after onset of illness or for 8 weeks in asymptomatic patients who traveled to areas  
7 with active ZIKV transmission <sup>10,11</sup>. This distinction between symptomatic and  
8 asymptomatic patients has, to our knowledge, no scientific basis to support that viral  
9 persistence in semen of asymptomatic patients is shorter than in symptomatic ZIKV  
10 infections. Further studies are needed to better characterize the frequency and  
11 duration of ZIKV persistence in semen. For other emerging viruses such as Ebola, the  
12 duration of the virus in semen has been expanding as long as new studies and larger  
13 series of patients have been analyzed, moving from 91 days in the Kikwit outbreak <sup>12</sup> to  
14 18 months in the West African epidemic <sup>13</sup>. Likewise, factors influencing viral  
15 persistence in semen should be identified for a better understanding of ZIKV kinetics in  
16 humans.

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EIMC-D-16-00345 REVISADO

TITLE: PERSISTENCE OF ZIKA VIRUS IN SEMEN 93 DAYS AFTER THE ONSET OF SYMPTOMS

TÍTULO: PERSISTENCIA DEL VIRUS ZIKA EN SEMEN 93 DÍAS TRAS EL COMIENZO DE LOS SÍNTOMAS

**Abstract**

**Introduction:** Zika virus is transmitted mainly through the bites of infected Aedes mosquitoes, although mother-to-child and sexual transmission have also been described. The presence of zika virus in semen after infection seems to be not uncommon, but the duration of viral persistence is not well determined.

**Methods:** We used molecular, serological and cell culture methods for the diagnosis and follow up of a case of zika virus infection imported from Venezuela. Serial samples of serum, urine and semen were analyzed to investigate zika virus persistence.

**Results:** zika virus was detected in semen samples up to 93 days after the onset of symptoms.

**Conclusions:** Our results confirm persistence of zika virus in semen samples for long periods after infection

## Resumen

**Introducción:** El virus zika se transmite fundamentalmente por la picadura de mosquitos Aedes infectados, aunque también es posible la transmisión de madre a hijo y la transmisión sexual. La presencia del virus Zika en semen tras la infección parece ser algo relativamente frecuente, pero la duración de la persistencia viral no es bien conocida.

**Métodos:** Mediante técnicas moleculares, serológicas y cultivo celular se diagnóstico un caso de zika importado de Venezuela y se tomaron muestra seriadas de suero, orina y semen para investigar la persistencia del virus

**Resultados:** El virus zika fue detectado en muestras de semen recogidas 93 días después del inicio de los síntomas

**Conclusiones:** Nuestros resultados confirman la persistencia del virus zika en semen por períodos prolongados de tiempo después de la infección

**Key words:** zika virus; semen; persistence

**Palabras clave:** virus zika; semen; persistencia

## Case report

1  
2  
3 In January 2016 a man in his 30s, previously diagnosed with prostatitis in December  
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6 2015 and in treatment with ciprofloxacin, presented to the Tropical Medicine  
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8 Department at Hospital Clinic (Barcelona) two days after his return from a 15-day  
9  
10 holiday trip to Venezuela. During his stay he reported self-limited hematospermia and  
11  
12 ceftibuten was added by a local urologist to his previous ciprofloxacin regime. In the  
13  
14 trip back to Europe he started with malaise, fever ( $\geq 39^{\circ}\text{C}$ ), myalgias, arthralgias and  
15  
16 retro-orbital pain. One day after, he developed a total itchy body macular rash,  
17  
18 followed by conjunctivitis. He had not been vaccinated for this trip. He had received  
19  
20 yellow fever vaccination in the past and referred dengue fever infection during his  
21  
22 childhood. Medical examination revealed bilateral enlarged latero cervical lymph  
23  
24 nodes, non purulent conjunctivitis and an intense itchy maculopapular rash diffused on  
25  
26 the face, trunk, arms and legs. He had mild transaminitis (alanine aminotransferase  
27  
28 195 UI/L, aspartate aminotransferase 79 UI/L) and elevated C-reactive protein (1.69  
29  
30 mg/dl). A complete blood count, blood levels of electrolytes, as well as coagulation and  
31  
32 renal-function tests were normal. Chest radiograph results were unremarkable. The  
33  
34 fever and the cutaneous rash resolved in 3 days while malaise, weakness, and myalgia  
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36 lasted for 1 week after presentation.  
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## Laboratory investigations

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55 Diagnosis of zika virus (ZIKV) infection was established by real time reverse-  
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57 transcription polymerase chain reaction (RT-PCR) using a commercial kit (RealStar Zika  
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59 Virus RT-PCR Kit 1.0, Altona Diagnostics, Hamburg, Germany) in a serum sample  
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1 collected three days post onset of illness (dpi). Dengue and chikungunya RNA were not  
2 detected by specific real-time RT-PCR assays and malaria was ruled out by negative  
3 blood thick and thin smears. The presence of ZIKV was confirmed by Sanger  
4 sequencing analysis of a 270 bp amplicon (from a generic flavivirus RT-PCR assay<sup>1</sup>) of  
5 the NS5 gene. The patient agreed to provide follow up samples to investigate the  
6 presence of ZIKV in semen, serum and urine samples. A summary of the samples  
7 collected and the results obtained is shown in Table 1. No ZIKV RNA was detected in  
8 follow up serum or urine samples. The first semen sample was obtained at 30 dpi and  
9 tested positive by ZIKV real time RT-PCR. Two more semen samples obtained at 51 and  
10 93 dpi also contained detectable levels of ZIKV RNA. A fourth semen sample collected  
11 201 dpi tested negative for ZIKV. For cell culture, semen samples were kept frozen  
12 until inoculated onto Vero cells and monitored for virus growth by visual inspection of  
13 the cytopathic effect and by RT-PCR of culture supernatants, but the virus could not be  
14 isolated. Serological tests were performed for dengue (Panbio ELISA, Alere, Australia),  
15 chikungunya and zika (Immunofluorescence, Euroimmun, Germany). The patient  
16 tested positive for IgG against dengue upon first presentation at the Hospital, in  
17 agreement with the dengue virus infection he reported to have suffered during his  
18 childhood. This first sample tested positive for IgG against zika by immunofluorescence  
19 but was negative by Anti-Zika Virus ELISA (Euroimmun, Germany), in concordance with  
20 the higher specificity reported for the ELISA test<sup>2</sup>. IgM against zika was positive one  
21 month after onset of symptoms and was undetectable 2 months later.

## Discussion

1  
2  
3 ZIKV is, to date, the only flavivirus transmitted through sexual contact, and this mode  
4  
5 of transmission might be more frequent than previously thought. So far, male to male  
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8 <sup>3</sup>, male to female <sup>4,5</sup> and female to male sexual transmission <sup>6</sup> have been documented  
9  
10 and up to 11 countries have reported ZIKV sexual transmission. While most cases have  
11  
12 been originated from men with previous history of ZIKV symptoms, the virus can also  
13  
14 be spread through sexual contact before the symptoms develop and even from  
15  
16 asymptomatic ZIKV infections <sup>7</sup>. ZIKV sexual transmission is of public health concern,  
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18 particularly for pregnant women given the possibility of ZIKV induced fetal  
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20 abnormalities <sup>8</sup>.  
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28 The duration of ZIKV persistence in semen is yet poorly understood. Reports of  
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30 travelers that acquired ZIKV during the present American epidemic showed viral  
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32 persistence in semen for 47, 62, 76 and 80 days. Recently, a case of Zika Virus  
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34 persistence for 6 months in semen has been described in a traveler returning from  
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36 Haiti to Italy<sup>9</sup>. In our case, ZIKV infection imported from Venezuela was confirmed  
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38 during the viremic period 3 days after the onset of symptoms. Results of the follow up  
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40 samples suggest that viral persistence was restricted to semen and longer shedding in  
41  
42 urine was not demonstrated. Although it is possible to isolate ZIKV from semen, our  
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44 attempts were not successful. Failure to isolate ZIKV from semen samples has been  
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46 reported in other studies<sup>9</sup>, with even higher viral loads (lower Ct) than in our case. The  
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48 RT-PCR Cycle Threshold (Ct) values obtained in the three positive semen samples  
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50 remained relatively stable until the last positive sample was tested as long as 93 days  
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52 after the onset of symptoms. Our results confirm longer periods for ZIKV presence in  
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1 semen and represent, to our knowledge, the first reported follow up of zika virus in  
2 semen in Spain. Unfortunately, samples between the third and the sixth month after  
3 symptomatic illness were not available for testing. We can only suggest that  
4 somewhere between 93 and 201 dpi ZIKV RNA declined to undetectable levels.  
5 Current recommendations include a precautionary period of safer sex/abstinence for 6  
6 months after onset of illness or for 8 weeks in asymptomatic patients who traveled to  
7 areas with active ZIKV transmission <sup>10,11</sup>. This distinction between symptomatic and  
8 asymptomatic patients has, to our knowledge, no scientific basis to support that viral  
9 persistence in semen of asymptomatic patients is shorter than in symptomatic ZIKV  
10 infections. Further studies are needed to better characterize the frequency and  
11 duration of ZIKV persistence in semen. For other emerging viruses such as Ebola, the  
12 duration of the virus in semen has been expanding as long as new studies and larger  
13 series of patients have been analyzed, moving from 91 days in the Kikwit outbreak <sup>12</sup> to  
14 18 months in the West African epidemic <sup>13</sup>. Likewise, factors influencing viral  
15 persistence in semen should be identified for a better understanding of ZIKV kinetics in  
16 humans.

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**Tablas (Tables)**

Laboratory tests	Days post onset of		
	3	30	48
<b>Real time RT-PCR</b>			
ZIKV (Serum)	Positive (28.7)		Negative
ZIKV (Urine)			Negative
ZIKV (Semen)		Positive (31.4)	Positive (32.58)
DENV (serum)	Negative		
CHIKV (serum)	Negative		
<b>Serology</b>			
IgM ZIKV	Negative	Positive	
IgG ZIKV	Positive (*)	Positive	
IgM DENV	Negative	Positive	
IgG DENV	Positive	Positive	
IgM CHIKV	Negative	Negative	
IgG CHIKV	Negative	Negative	