The use of non-invasive clinical specimens as a tool for epidemiological surveillance of Zika virus

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Oswaldo Cruz (1872-1917)

Oswaldo Cruz Foundation
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Publications: >1,500 papers per year

Teaching: >7 thousand students

Production:
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>9 million diagnostic kits
>4 billion medicines
>17 million biopharmaceuticals
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Environment and Health Promotion
Information and Communication
History of Science and Health
Planning and Management
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- Belo Horizonte
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- Curitiba
- Brasília

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International Cooperation

- Technical cooperation
- CPLP
- DNDI
- IBSA
- Pasteur Network (RIIP)
The Reference Laboratories hosted by Fiocruz

24 Diseases
50 Reference Laboratories
7 Outpatient clinics
Algorithm for testing of microcephaly suspected samples

Source: Ministry of Health, Brazil
Flavivirus Laboratory
Regional Reference Center for Dengue, Zika, Yellow Fever and Chikungunya diagnosis

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LABFLA
FIOCRUZ

ZIKAlliance

ZIKAction

WHO

ZikaPLAN

CIHR IRSC
Zika virus in Brazil

- Whole viral genome sequencing

Figure 1: Comparative genome BLAST Atis diagram of Zika virus
The green outer circle corresponds to the complete Brazilian Zika virus genome isolated from the amniotic fluid of patient 1. 10,753 bases were sequenced. The red circle corresponds to the Senegal (KJ331133.1) strain of Zika virus and the blue circle corresponds to the Uganda strain (NC_012532.1). The percentage deviation in GC content between the Brazilian Zika virus and the reference genome is represented along the Zika virus genome by the varying heights of the black bars. The innermost (black) circle corresponds to the reference genome (French Polynesia, KJ776701.1). Genomes shared identity between each strain and the reference genome are shown as percentages. BLAST-based local alignment search tool.

Figure 4: Maximum likelihood phylogeny of Brazilian Zika virus, other Flaviviridae genomes, and an alphavirus genome
Brazilian Zika virus (in red) was isolated from the amniotic fluid of patient 1, whose fetus was diagnosed with microcephaly. Approximate likelihood-ratio test and Bayesian inference support values are shown at nodes.
Chikungunya is an alphavirus alloother viruses are from the Flaviviridae family. DENV=dengue virus, JEV=Japanese encephalitis virus, YFV=yellow fever virus. ZIKV=Zika virus.
Zika virus surveillance in the state of Rio de Janeiro

Flavivirus Laboratory – FIOCRUZ

Samples from suspected ZIKV cases, 2015-2016

ZIKV detection rate: 29%

ZIKV detection rate: 38%

2015

2016*
Zika virus surveillance in the state of Rio de Janeiro

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9903 ZIKV suspected samples

3829 positive samples by RT-PCR (34%)

6074 negative samples by RT-PCR

2191 (+) serum samples

1416 (+) urine samples

63 (+) saliva samples

159 (+) other clinical samples
# Zika virus surveillance in the state of Rio de Janeiro

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Days after the first symptoms</th>
<th>Result RT-qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>0-18 days</td>
<td>Positive</td>
</tr>
<tr>
<td>Saliva</td>
<td>4 - 9 days</td>
<td>Positive</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>20 weeks</td>
<td>Positive</td>
</tr>
<tr>
<td>Breast milk</td>
<td>3- 20 days</td>
<td>Positive</td>
</tr>
<tr>
<td>Semen</td>
<td>4 - 16 days</td>
<td>Positive</td>
</tr>
<tr>
<td>Vaginal secretion</td>
<td>3- 23 days</td>
<td>Positive</td>
</tr>
<tr>
<td>Cerebrospinal fluid (CSF)</td>
<td>4-9 days</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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Zika virus surveillance in the state of Rio de Janeiro

Molecular diagnosis

Gold standard for molecular diagnosis of ZIKV
## Preliminary results

<table>
<thead>
<tr>
<th></th>
<th>No of positive samples (real-time RT-PCR)</th>
<th>Average day post infection</th>
<th>Ct value range (real-time RT-PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>16</td>
<td>3\textsuperscript{rd}</td>
<td>26 - 37</td>
</tr>
<tr>
<td>Saliva</td>
<td>13</td>
<td>5\textsuperscript{th}</td>
<td>28 - 38</td>
</tr>
<tr>
<td>Urine</td>
<td>16</td>
<td>8\textsuperscript{th}</td>
<td>28 - 38</td>
</tr>
<tr>
<td>TOTAL</td>
<td>45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Preliminary results

Percentage of ZIKV RNA detection in serum, urine and saliva

![Pie chart showing the percentage of ZIKV RNA detection in serum, urine, and saliva.](chart.png)
Preliminary results

Correlation between the type of clinical specimens and ZIKV detection
Preliminary results

Correlation between days after initial onset of symptoms and ZIKV RNA detection in serum
Preliminary results

Correlation between days after initial onset of symptoms and ZIKV RNA detection in urine
### Persistence of Zika Virus in Body Fluids — Preliminary Report


<table>
<thead>
<tr>
<th>Clinical specimen</th>
<th>Range of days of ZIKV RNA detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>14-54</td>
</tr>
<tr>
<td>Urine</td>
<td>8-39</td>
</tr>
<tr>
<td>Semen</td>
<td>34-81</td>
</tr>
</tbody>
</table>
Final considerations:

✓ Urine and saliva are useful non-invasive clinical specimens for ZIKV RNA detection;

✓ Prolonged ZIKV RNA detection in urine and saliva samples are important features for diagnosis and prevention of ZIKV;

✓ ZIKV RNA detection rates range from 30 to 37% in serum, urine and saliva samples;

✓ ZIKV RNA has been detected in serum, urine, saliva, semen, breast milk, amniotic fluid, vaginal secretion and CSF;

✓ The use of non-invasive clinical specimens collection for ZIKV diagnosis means an extra tool for epidemiological surveillance.

✓ Whole genome sequencing of viral RNA from different clinical specimen of one single patient will shed light into viral compartmentalization.
Thank you