Inactivation and Disinfection of Zika Virus in the Presence and Absence of Blood

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Microbac Laboratories, Inc.
Siena Duomo: Monument to Ambition... and Grief
### Some Worst Killer Plaques in History...

<table>
<thead>
<tr>
<th>Disease</th>
<th>Time</th>
<th>Location</th>
<th>Death toll</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Death</td>
<td>1340 - 1771</td>
<td>Europe, ME, Russia</td>
<td>75 m*</td>
<td><em>Yersinia pestis</em> bacteria</td>
</tr>
<tr>
<td>Smallpox</td>
<td>? - 1979</td>
<td>Global</td>
<td>300 m**</td>
<td><em>Variola</em> virus</td>
</tr>
<tr>
<td>“Spanish Flu”</td>
<td>1918 - 1919</td>
<td>Europe</td>
<td>50-100 m</td>
<td><em>Influenza (high path)</em> virus</td>
</tr>
<tr>
<td>Malaria</td>
<td>1600 - today</td>
<td>Tropical area</td>
<td>2 mil / yr</td>
<td><em>Plasmodium</em> parasite</td>
</tr>
<tr>
<td>AIDS</td>
<td>1981 - today</td>
<td>Global</td>
<td>30 m</td>
<td>HIV virus</td>
</tr>
</tbody>
</table>

* 30-60% death rate in Europe
** 80-90% native Americans died

The reason for the disappearance of black death is not exactly clear.

To date, there is still no cure for flu
Emerging and Re-emerging Infectious Diseases

Emerging infections are “infections that have newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic range” (Mores & Fauci, 2004).

- Examples: HIV; SARS; Zika virus

Re-emerging infections have been experienced previously but have reappeared in a more virulent form or in a new epidemiological setting

- Examples: Influenza A pandemics of 1918, 1957, and 1968

“Deliberately emerging” infectious diseases: from deliberate human actions

- Examples: Anthrax bioterrorist attack in the U.S.
Emerging/Re-emerging Viral Diseases

- Viruses constitute ~14% of known human pathogens, but ~75% of the pathogens discovered since 1980 are viruses.
- The first human virus discovered in 1901; to date there are 219 human viral species.
- Approximately 75% of emerging diseases are caused by RNA viruses.
- 73% (130/177) of emerging pathogens are zoonotic in origin.
- Most of emerging viruses are transmitted through mucosal and respiratory routes.

A warning from the WHO:

- the source of the next human pandemic is likely to be zoonotic and that wildlife is a prime culprit.
It is estimated that ~16% of all cancers are directly or indirectly associated with a microbial agent.
Experience from History…

- Quarantine
- *Travel restriction*
- *Hygiene*
- *Vector control*
- *Clean air, water, food...*
Zika Virus (ZIKV)

- *Flaviviridae* family, genus *Flavivirus*
- Enveloped; (+) ssRNA
- First discovered in 1947 and named after the Zika Forest in Uganda. In 1952, the first human cases of Zika were detected.
- Occurrences of ZIKV infection were reported in tropical Africa, Southeast Asia, and the Pacific Islands prior to 2015. But it was not until early 2016 that ZIKV was linked to severe *birth defects* such as microcephaly.
- Other mosquitoes-transmitted Flaviviruses such as Yellow Fever, Dengue Fever, Japanese encephalitis, West Nile virus, etc. may lead to more severe fever symptoms; but they are *not* considered to lead to teratogenic effects.

- Is ZIKV unique among flaviviruses?
- *What happened to ZIKV in 2015 - 2016? Or that the teratogenic effects previously existed but were missed?*
Combating ZIKV…

Battle against ZIKV

Epidemiology / Clinical

Vaccines / Therapeutics

Pathogenesis / Immune Response / Animal model

Mosquito control

Hygiene / Infection Control

Research

Intervention
# Routes of Viral Transmission

**Routes of transmission of human viruses:**

- **Arthropods** (mosquitoes, ticks, etc.)
- **Vertical / Parental**
- **Sex**
- **Blood**
- **Zoonosis**
  - ~70% of human pathogenic viruses are zoonotic origin
- **Air / Respiratory**
- **Food**
- **Water**
- **Environmental contact**
ZIKV Infection Control: Questions

- How long can ZIKV survive in the environment?
- Is ZIKV stable under heat, low pH, and high pH?
- Is ZIKV sensitive to commonly used disinfectants / antiseptics, such as Chlorine, PAA, Alcohol, and Quats?
- Does organic load in the matrix, such as blood, affect the stability or susceptibility of ZIKV?
- What happens to the ZIKV RNA when virus is inactivated?
- How does ZIKV compare to other viruses?
ZIKV Sustainability upon Drying

Survival of ZIKV upon drying on hard surface

Time post drying (hr)

Titer (Log_{10} TCID_{50}/mL)

ZIKV infectivity in 5% serum
ZIKV Survives Longer in Blood

Survival of ZIKV upon drying on hard surface in blood or 5% serum medium

Time post drying (hr)

Titer (Log_{10} TCID_{50}/mL)

- virus in 5% serum
- virus in 90% blood

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Methods for Viral Inactivation

**Physical**
- Heat
- HTST
- UV
- Gamma
- Electron beam
- High pressure

**Aldehydes**
- Glutaraldehyde
- Formaldehyde
- OPA

**Peroxygens**
- $\text{H}_2\text{O}_2$
- Peracetic Acid
- Ozone

**Halogens**
- Chlorine
- Iodine

**Physical**
- Heat
- HTST
- UV
- Gamma
- Electron beam
- High pressure

**Aldehydes**
- Glutaraldehyde
- Formaldehyde
- OPA

**Peroxygens**
- $\text{H}_2\text{O}_2$
- Peracetic Acid
- Ozone

**Halogens**
- Chlorine
- Iodine

**Alcohols**
- Ethanol
- IPA

**Quats**
- Low pH
- High pH

**Phenolics**
- Antiviral Drugs
- Biguanides
  - Chlorhexidine
  - PHMB
- Bisphenols
  - Triclosan
- Solvent/Detergent

**Heavy metals**
- Silver
- Copper
- Zinc
Antiviral Products: Mechanisms of Action

- Damaging viral envelope, capsid, proteins, and/or nucleic acid

- Blocking cellular receptors

- Blocking viral surface proteins

- Inhibiting viral replication

- Blocking viral packaging/release

- Promoting cell survival / growth

- Modulating host immune system - in vivo
Experimental Design

Glass Petri dish → Dry virus onto carrier → Apply disinfectant or heat → Hold for contact time; then add neutralizer → Scrape off mixture; assay for virus

Mix virus with disinfectant in solution or directly go to heat → Add neutralizer; mix → Virus infectivity by TCID50 → Viral RNA by qRT-PCR

Uninfected Vero E6 cells → 10 d post-infection
ZIKV in 5% serum is Sensitive to Chlorine

Virus Infectivity Reduction (Log_{10})

**Sodium Hypochlorite (5% serum)**

<table>
<thead>
<tr>
<th>Exposure (contact) time</th>
<th>500 ppm chlorine</th>
<th>2000 ppm chlorine</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 sec</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1 min</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2 min</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>5 min</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

* A complete inactivation of virus was achieved in all cases.
Blood is protective of ZIKV against chlorine

Sodium Hypochlorite (90% blood)

* A complete inactivation of virus was achieved at 10,000 ppm only.
Virus Infectivity Reduction (Log$_{10}$)

Efficacy of PAA on ZIKV is also Organic-dependent

Peracetic Acid (PAA)

- A complete inactivation of virus was achieved at 5% serum.
ZIKV is Sensitive to Alcohol and Quats w/ or w/o Blood

70% Isopropyl Alcohol

Note: A complete inactivation of virus was achieved in all cases.

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In general, an enteric flavivirus such as BVDV can be considered a worst case for low pH for env viruses, and a worst case for high pH for all viruses. Parvoviruses are not worst case.
Heat Inactivation of ZIKV

Heat (56°C, 2 hr)

Virus Infectivity Reduction (Log₁₀)

Exposure time (min)

- Virus in 5% serum
- Virus in 90% blood

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* A complete inactivation of virus was achieved in IPA and Quats under 5% serum and 90% blood.
Infectivity loss vs. RNA damage

Reduction of ZIKV infectivity and RNA after various treatments

* A complete inactivation of virus was achieved in chlorine and PAA under 5% serum.
How Unique is ZIKV within *Flaviviruses*?

- *How does ZIKV compare to other flaviviruses?*
# Flaviviridae

<table>
<thead>
<tr>
<th>Genus</th>
<th>Host</th>
<th>Example virus</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flavivirus</strong></td>
<td>Humans, Mammals, Mosquitos, Ticks</td>
<td><strong>Zika virus</strong></td>
<td>mosquito-borne</td>
</tr>
<tr>
<td></td>
<td>Humans</td>
<td><strong>Dengue Fever virus</strong></td>
<td>mosquito-borne</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Japanese Encephalitis virus</strong></td>
<td>mosquito-borne</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>St. Louis Encephalitis virus</strong></td>
<td>mosquito-borne</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>West Nile virus</strong></td>
<td>mosquito-borne</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Yellow Fever virus</strong></td>
<td>mosquito-borne</td>
</tr>
<tr>
<td><strong>Hepacivirus</strong></td>
<td>Humans</td>
<td><strong>Hepatitis C virus</strong></td>
<td>sex, blood</td>
</tr>
<tr>
<td><strong>Pegivirus</strong></td>
<td>Mammals</td>
<td><strong>GB virus A</strong></td>
<td>unknown</td>
</tr>
<tr>
<td><strong>Pestivirus</strong></td>
<td>Mammals</td>
<td><strong>Bovine Viral Diarrhea virus</strong></td>
<td>Vertical (parental)</td>
</tr>
</tbody>
</table>

*first human virus discovered in 1901 by Walter Reed*
# Comparison of Susceptibility of ZIKV, BVDV & WNV

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Contact time</th>
<th>Log₁₀ reduction in infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ZIKV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5% serum</td>
</tr>
<tr>
<td>pH 4</td>
<td>5 min</td>
<td>0.9 ± 0.0</td>
</tr>
<tr>
<td>pH 10</td>
<td>5 min</td>
<td>1.4 ± 0.0</td>
</tr>
<tr>
<td>70% IPA</td>
<td>15 sec</td>
<td>≥ 5.1</td>
</tr>
<tr>
<td>Quat/Alcohol</td>
<td>15 sec</td>
<td>≥ 3.5</td>
</tr>
<tr>
<td>500 ppm chlorine</td>
<td>5 min</td>
<td>≥ 4.1</td>
</tr>
<tr>
<td>2000 ppm chlorine</td>
<td>5 min</td>
<td>≥ 4.1</td>
</tr>
<tr>
<td>1000 ppm PAA</td>
<td>5 min</td>
<td>≥ 4.9</td>
</tr>
<tr>
<td>Heat (56°C)</td>
<td>20 min</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

≥ Denotes complete inactivation of virus
How Unique is ZIKV Compared to Other Viruses?

➢ How does ZIKV compare to non-flaviviruses?
The “Hierarchy” of Micro-organism Resistance

- Prions
- Spores/oocysts
- Small non-env viruses
- Mycobacteria
- Large non-enveloped viruses
- Mold
- Vegetative bacteria, yeast
- Enveloped viruses

Resistance
## Comparing ZIKV and Other Viruses

<table>
<thead>
<tr>
<th>DNA</th>
<th>Enveloped</th>
<th>Non-enveloped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>herpes simplex virus</strong></td>
<td>adenovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>simian virus 40</td>
</tr>
<tr>
<td>RNA</td>
<td><strong>influenza virus</strong></td>
<td>BVDV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>poliovirus</td>
</tr>
<tr>
<td></td>
<td><strong>ZIKV</strong></td>
<td>feline calicivirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(norovirus)</td>
</tr>
</tbody>
</table>
## Comparing ZIKV and Other Viruses for Heat Resistance

<table>
<thead>
<tr>
<th>Virus:</th>
<th>BVDV</th>
<th>HSV-1</th>
<th>Influenza</th>
<th>ZIKV</th>
<th>Adenovirus 5</th>
<th>FCV</th>
<th>Poliovirus</th>
<th>SV40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat condition:</td>
<td>46C</td>
<td>56C</td>
<td>65C</td>
<td></td>
<td>5 min</td>
<td>20 min</td>
<td>60 min</td>
<td>2 hr</td>
</tr>
<tr>
<td>Matrix / Carrier:</td>
<td>Glass surface</td>
<td>Stainless steel</td>
<td>Liquid - 5% serum</td>
<td>Liquid - 100% serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Heat Inactivation of ZIKV on Surface vs. in Liquid

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Contact time</th>
<th>Glass</th>
<th>Steel</th>
<th>Medium</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>46°C</td>
<td>20 min</td>
<td>1.4</td>
<td>0.4</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>1.7</td>
<td>1.0</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>0.5</td>
<td>0.7</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>56°C</td>
<td>20 min</td>
<td>1.3</td>
<td>1.8</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>2.0</td>
<td>2.6</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>0.7</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>65°C</td>
<td>20 min</td>
<td>2.8</td>
<td>3.4</td>
<td>3.8</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>3.7</td>
<td>4.1</td>
<td>≥ 4.3</td>
<td>≥ 4.2</td>
</tr>
</tbody>
</table>

≥ Denotes complete inactivation of virus

* Results represent the average from two independent experiments
Heat Inactivation of Virus: D Value

$D = a \cdot \text{temperature}^{-b}$

$\text{temperature (°C)} = \left(\frac{D}{a}\right)^{-\frac{1}{b}}$

Threshold temperature for capsid opening

Most capsids already open

$D$ (min for 1-log$_{10}$ inactivation)

Temperature (°C)
Relationship between D and temperature

**HSV-1 relationship between D and temperature**

**Glass**
- Equation: \( y = 3E+09x^{4.87} \)
- \( R^2 = 0.9329 \)

**Steel**
- Equation: \( y = 2E+09x^{4.743} \)
- \( R^2 = 0.9578 \)
### Viruses Exhibit Different Resistance to Heat

<table>
<thead>
<tr>
<th>Virus</th>
<th>Env</th>
<th>Temp.</th>
<th>Glass</th>
<th>Steel</th>
<th>Medium</th>
<th>Serum</th>
<th>Ave.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>Y</td>
<td>65°C</td>
<td>6.0</td>
<td>4.8</td>
<td>4.8</td>
<td>4.2</td>
<td>5.0</td>
</tr>
<tr>
<td>PV-1</td>
<td>N</td>
<td>65°C</td>
<td>4.0</td>
<td>4.4</td>
<td>3.9</td>
<td>9.3</td>
<td>5.4</td>
</tr>
<tr>
<td>AD5</td>
<td>N</td>
<td>65°C</td>
<td>u.d.</td>
<td>6.0</td>
<td>5.6</td>
<td>5.1</td>
<td>5.6</td>
</tr>
<tr>
<td>ZIKV</td>
<td>Y</td>
<td>65°C</td>
<td>7.2</td>
<td>6.0</td>
<td>5.5</td>
<td>5.3</td>
<td>6.0</td>
</tr>
<tr>
<td>FCV</td>
<td>N</td>
<td>65°C</td>
<td>6.7</td>
<td>6.7</td>
<td>6.5</td>
<td>6.3</td>
<td>6.6</td>
</tr>
<tr>
<td>FLU</td>
<td>Y</td>
<td>65°C</td>
<td>13.2</td>
<td>9.4</td>
<td>9.6</td>
<td>9.5</td>
<td>10.4</td>
</tr>
<tr>
<td>BVDV</td>
<td>Y</td>
<td>65°C</td>
<td>7.5</td>
<td>8.1</td>
<td>13.1</td>
<td>15.0</td>
<td>10.9</td>
</tr>
<tr>
<td>SV40</td>
<td>N</td>
<td>65°C</td>
<td>19.8</td>
<td>18.9</td>
<td>12.7</td>
<td>14.4</td>
<td>16.5</td>
</tr>
</tbody>
</table>
Proposed Mechanism of Heat Inactivation on Virus

- **Intact virion**
  - Capsid
  - Genome

- **Heat**
  - Leaky capsid
  - Empty capsid

- **Nuclease degradation**
  - Free genomic material

---

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The “Hierarchy” is Not Always True

- prions
- spores/oocysts
- small non-env viruses
- mycobacteria
- large non-enveloped viruses
- mold
- vegetative bacteria, yeast
- enveloped viruses

Resistance
• ZIKV in blood remains highly infectious after 8 hours dried

• ZIKV is sensitive to alcohol and quats with or without blood

• The efficacy of Chlorine and PAA is influenced by blood

• ZIKV is stable at pH 4 and pH 10

• The blood matrix shall be considered when selecting a disinfectant

• Be cautious of generalities about efficacy of heat on viruses

• The organism “hierarchy” is useful, but not always true
Acknowledgement

Cameron Wilde
Zheng Chen
Tanya Kapes
Semhar Fanuel
Cory Chiossone
Salimatu Lukula

Donna Suchmann
Raymond Nims
Emily Huang
Laura Rowson