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

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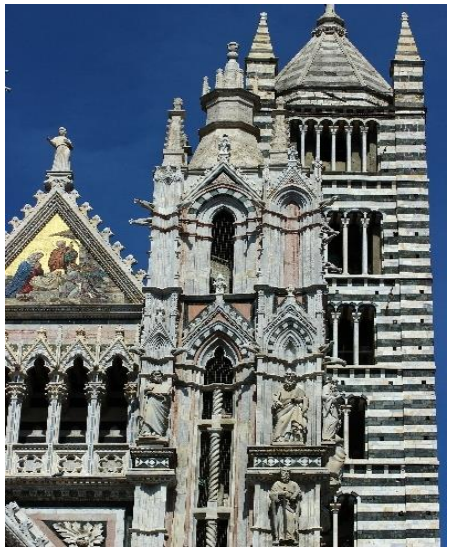
Siena Duomo: Monument to Ambition… and Grief



Some Worst Killer Plaques in History…

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

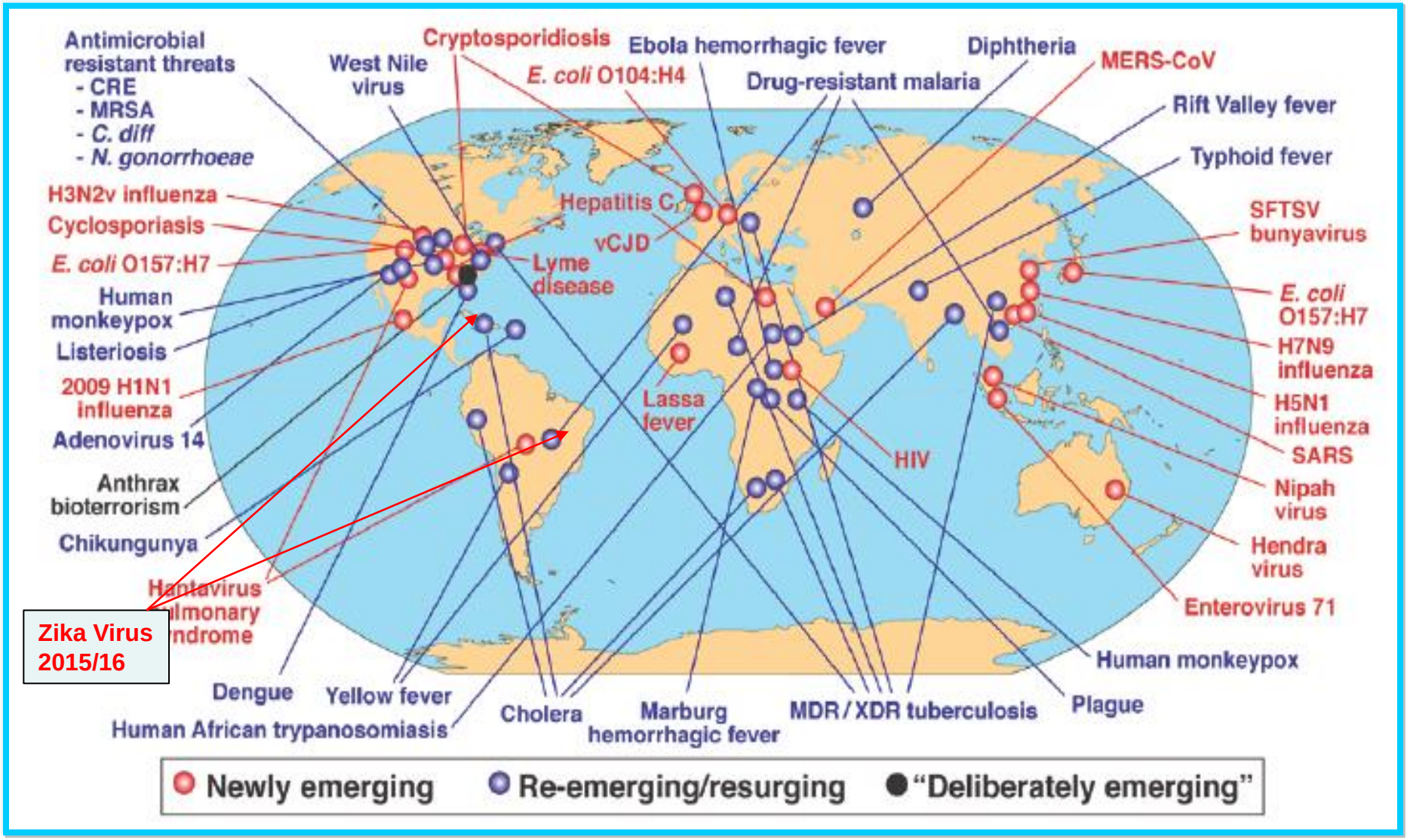
** 30-60% death rate in Europe*

*** 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



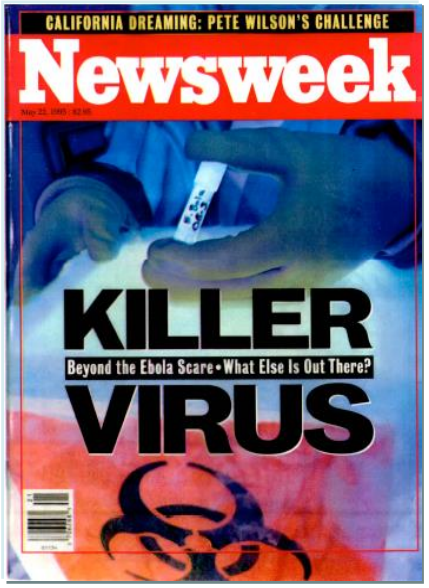
Morens & Fauci In IOM (Institute of Medicine). 2015. Emerging Viral Diseases: The One Health Connection.

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

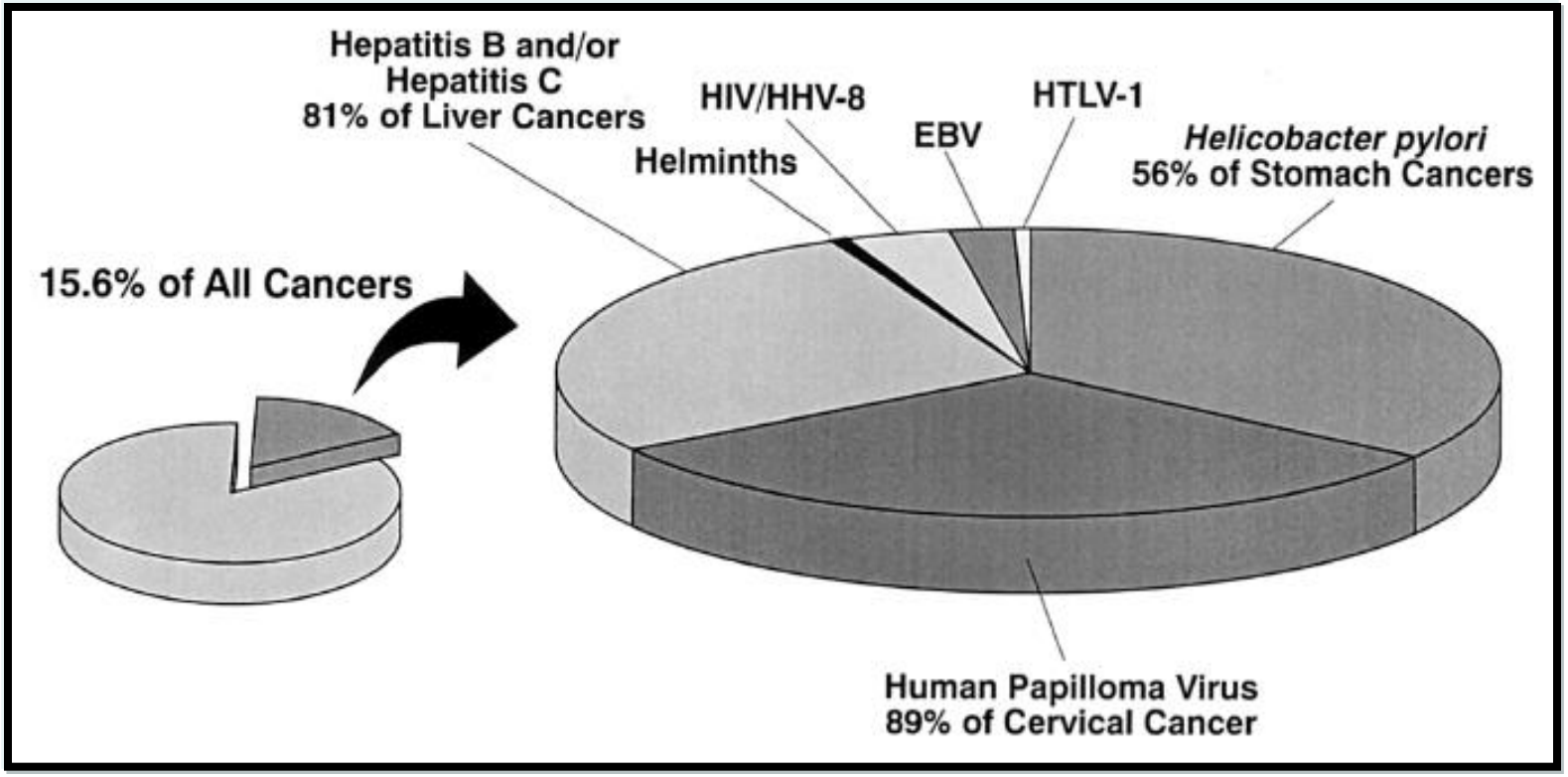


World Health Organization

A warning from the WHO:

- the source of the next human pandemic is likely to be zoonotic and that wildlife is a prime culprit

Infectious Causes of Cancer



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)

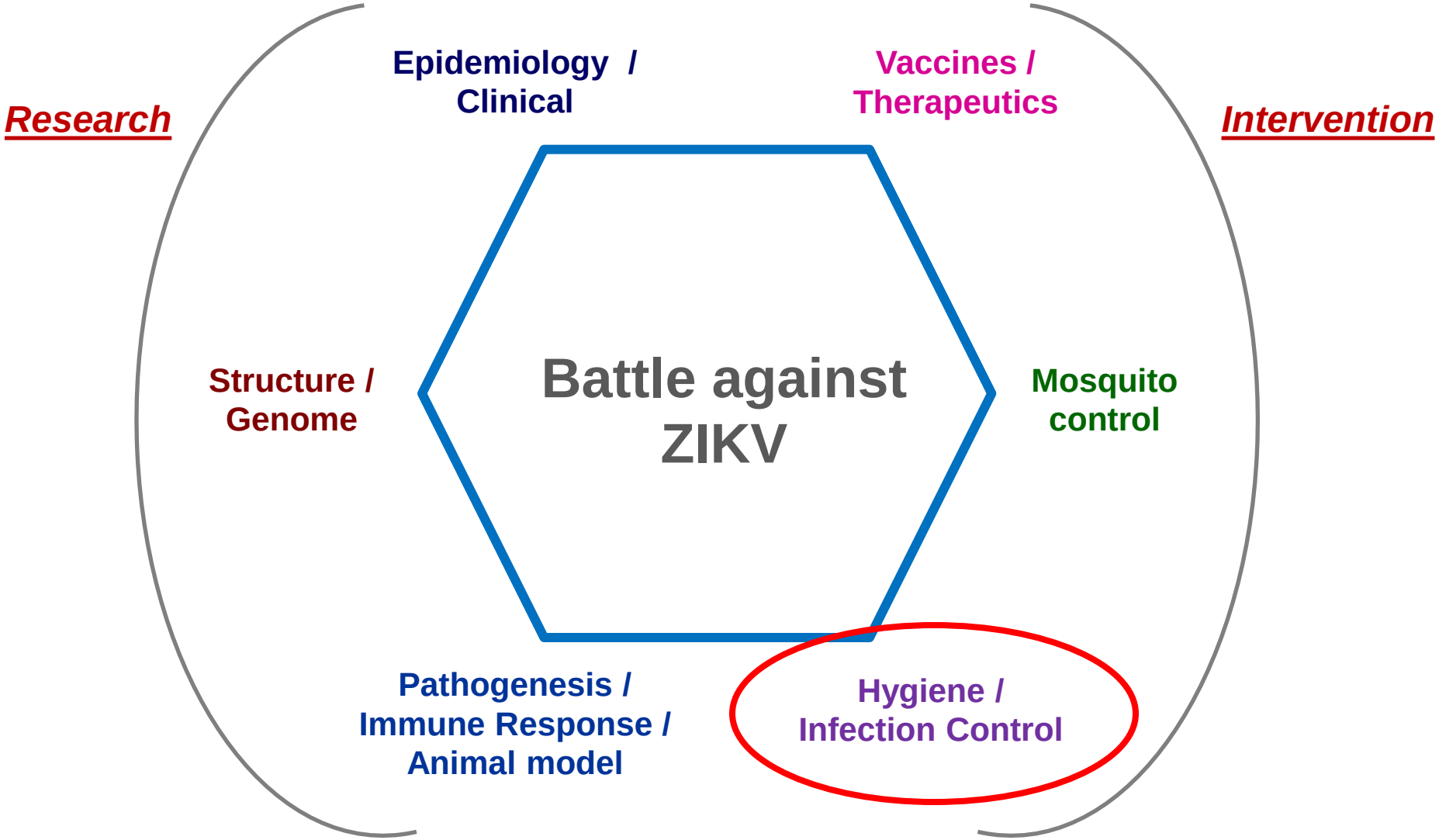
- ***Flaviviridae*** **family, genus** ***Flavivirus***
- **Enveloped; (+) ssRNA**
- **First discovered in 1947 and named after the Zika Forest in Uganda. In 1952, the first human cases of Zika were detected.**
- **Occurrences of ZIKV infection were reported in tropical Africa, Southeast Asia, and the 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?*

Zika Cases in the World

https://www.cdc.gov/zika/geo/active-countries.html

Combating ZIKV…



Routes of Viral Transmission

Routes of transmission of human viruses:

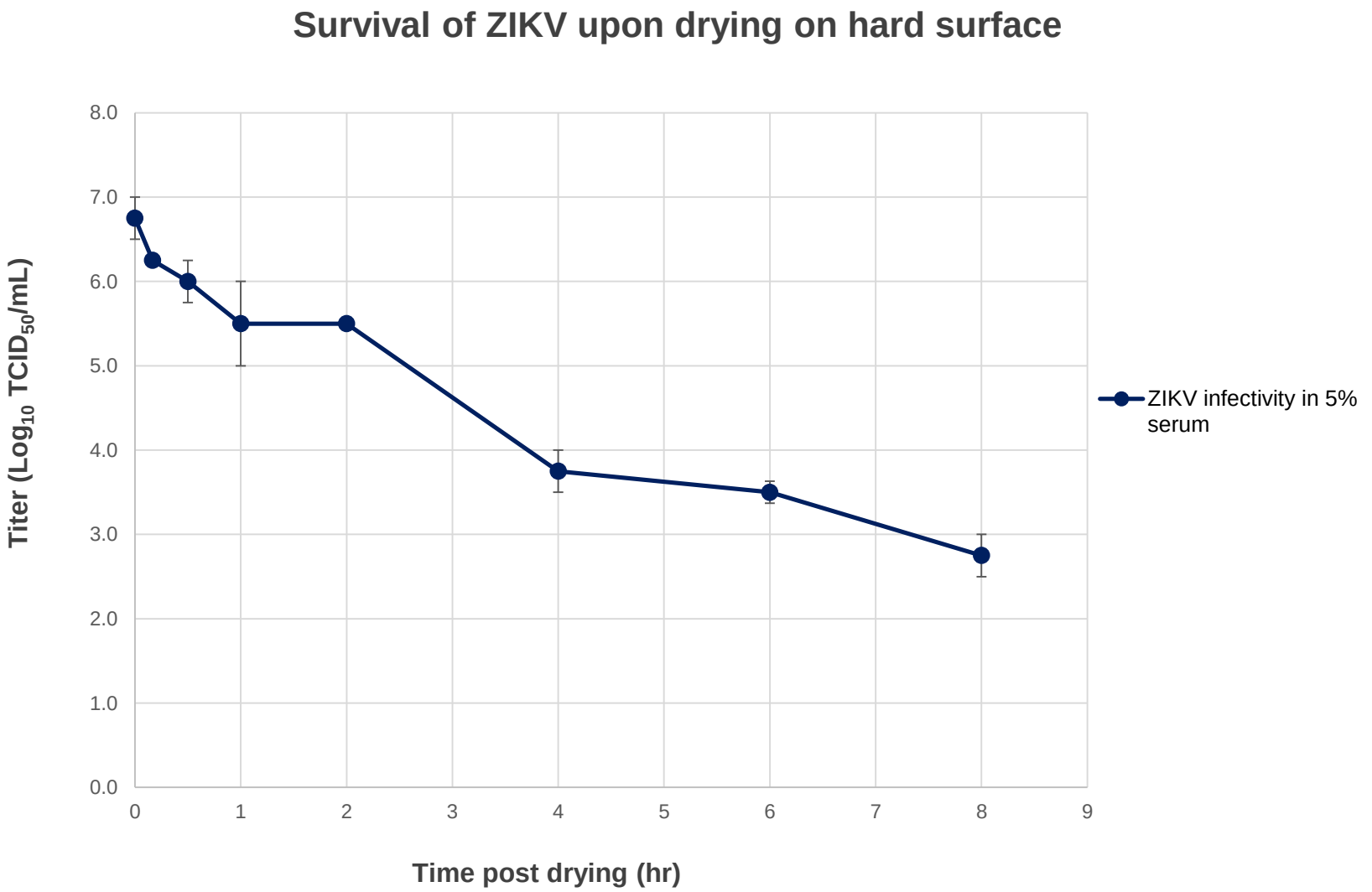
Possible routes for ZIKV

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

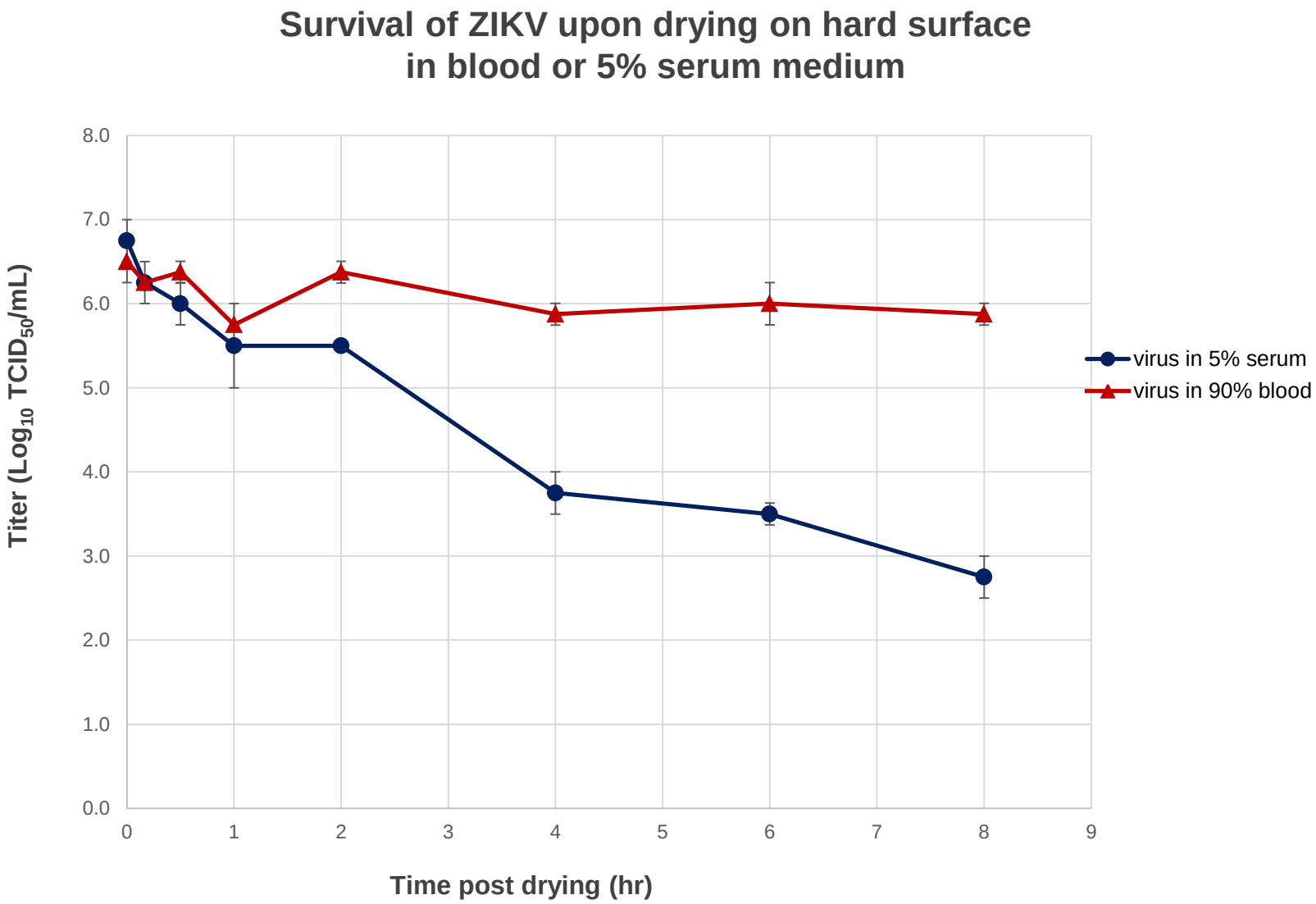
ZIKV Infection Control: Questions

- 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



ZIKV Survives Longer in Blood



Methods for Viral Inactivation

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

Aldehydes
- Glutaraldehyde
- Formaldehyde
- OPA

Peroxygens
- H_2O_2
- Peracetic Acid
- Ozone

Halogens
- Chlorine
- Iodine

Alcohols
- Ethanol
- IPA

Quats

Low pH

High pH

Phenolics

Heavy metals
- Silver
- Copper
- Zinc

Biguanides
- Chlorhexidine
- PHMB

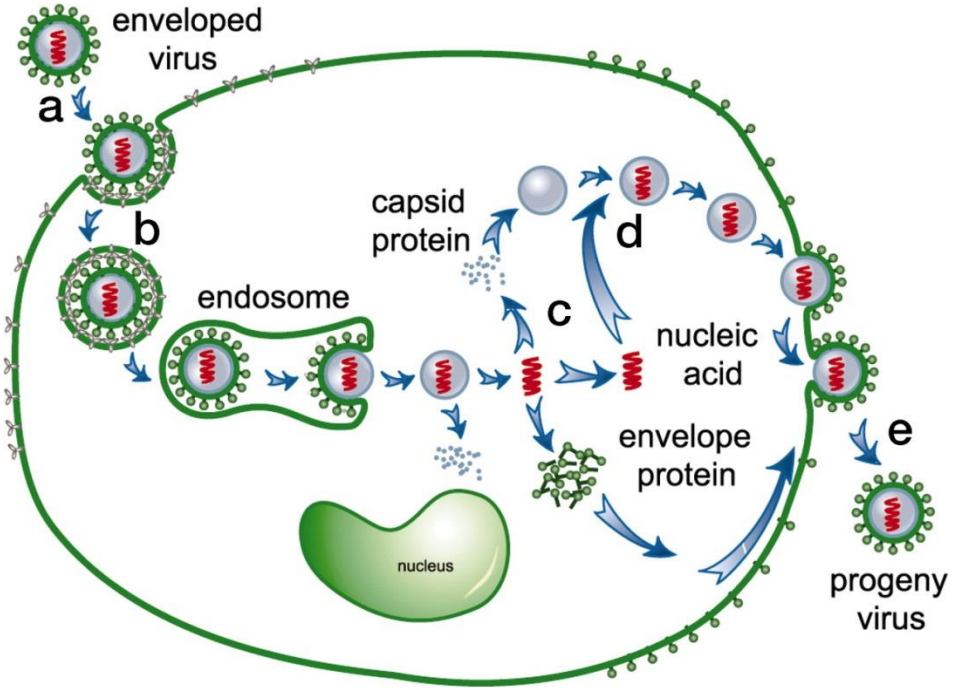
Bisphenols
- Triclosan

Solvent/Detergent

Antiviral Drugs

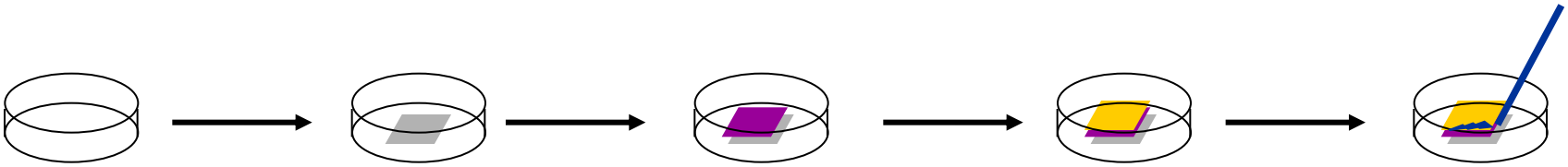
Antiviral Products: Mechanisms of Action

- *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



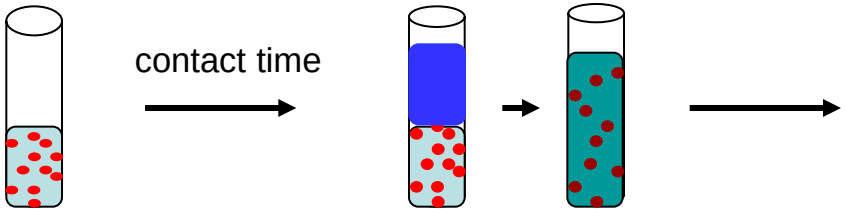
Glass Petri dish

Dry virus onto carrier

Apply disinfectant or heat

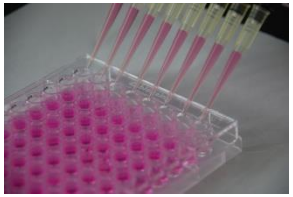
Hold for contact time; then add neutralizer

Scrape off mixture; assay for virus



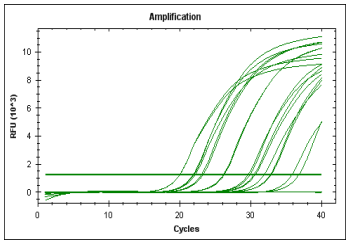
Mix virus with disinfectant in solution or directly go to heat

Add neutralizer; mix



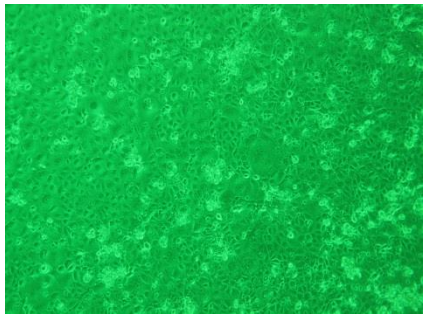
virus infectivity by TCID50

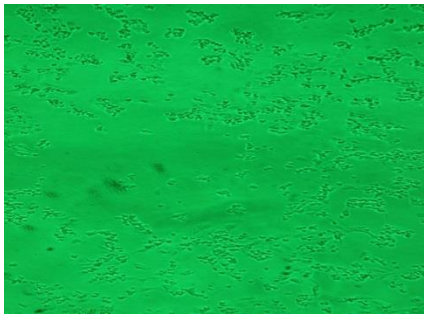
+



viral RNA by qRT-PCR

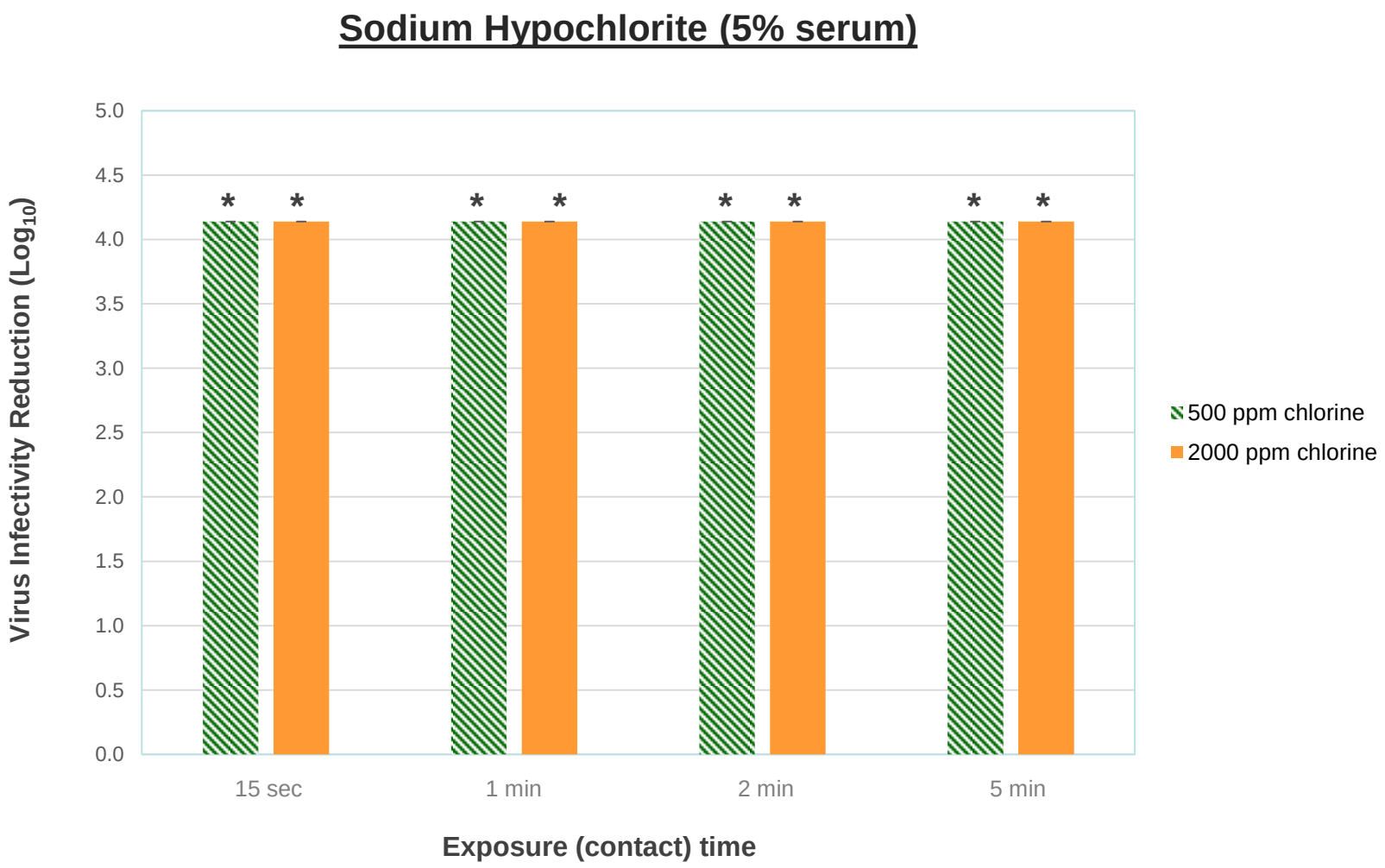
Uninfected Vero E6 cells





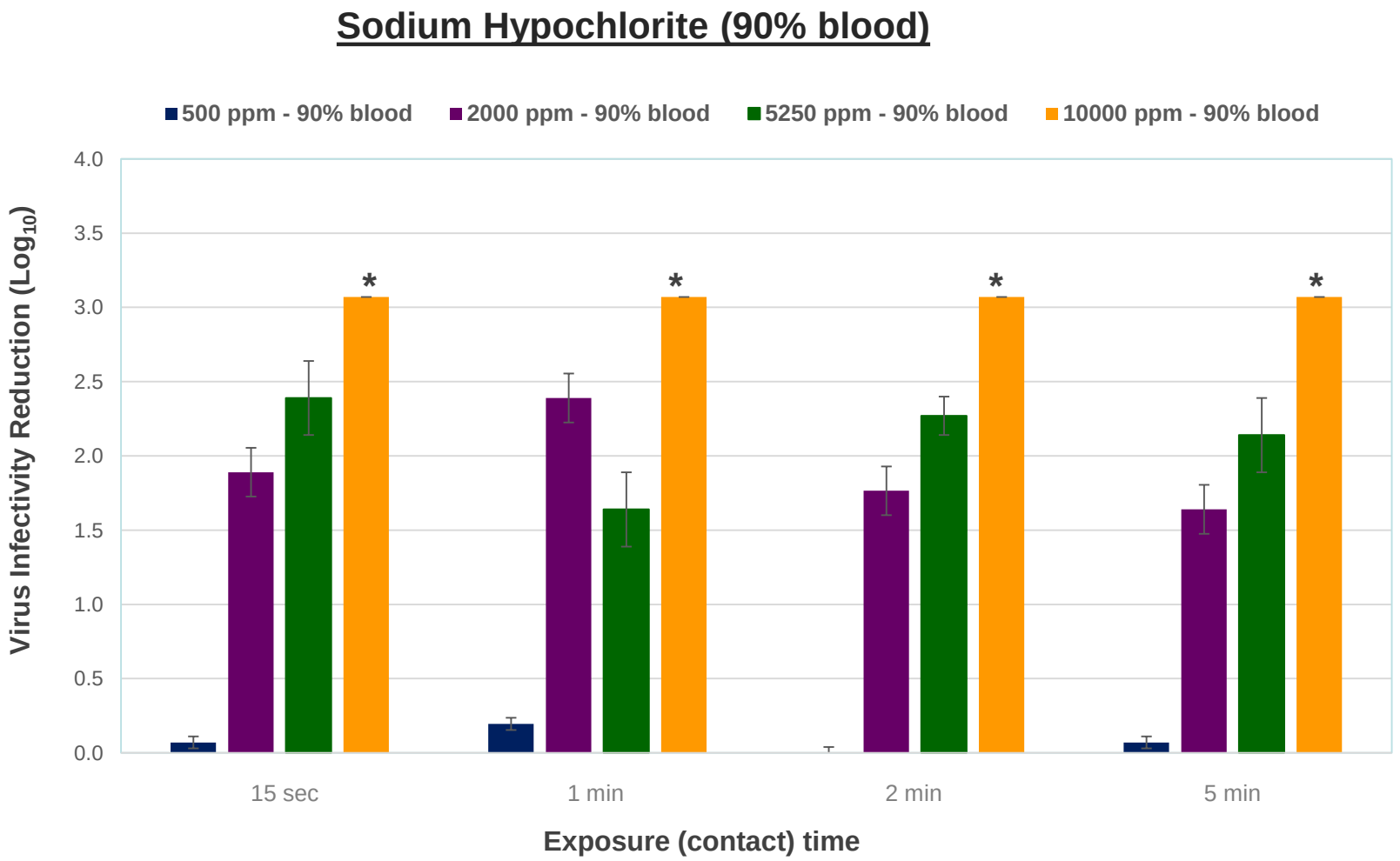
10 d post-infection

ZIKV in 5% serum is Sensitive to Chlorine



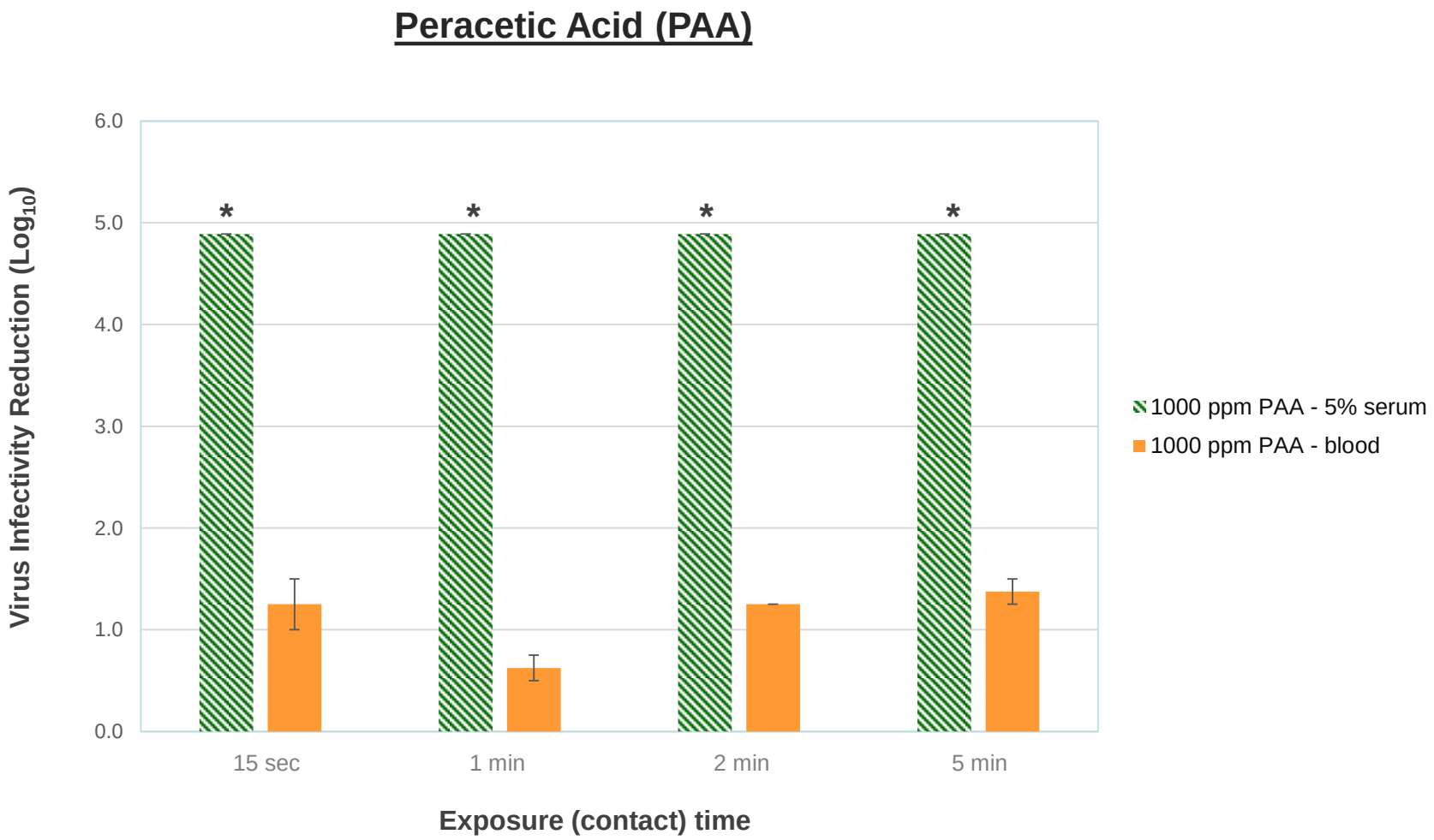
* A complete inactivation of virus was achieved in all cases.

Blood is protective of ZIKV against chlorine



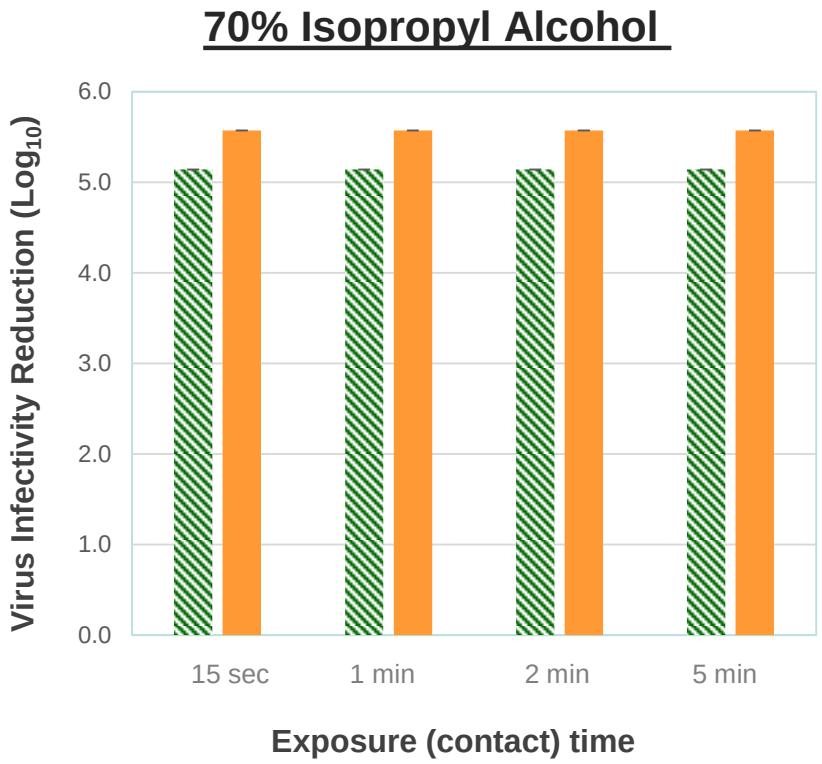
* A complete inactivation of virus was achieved at 10,000 ppm only.

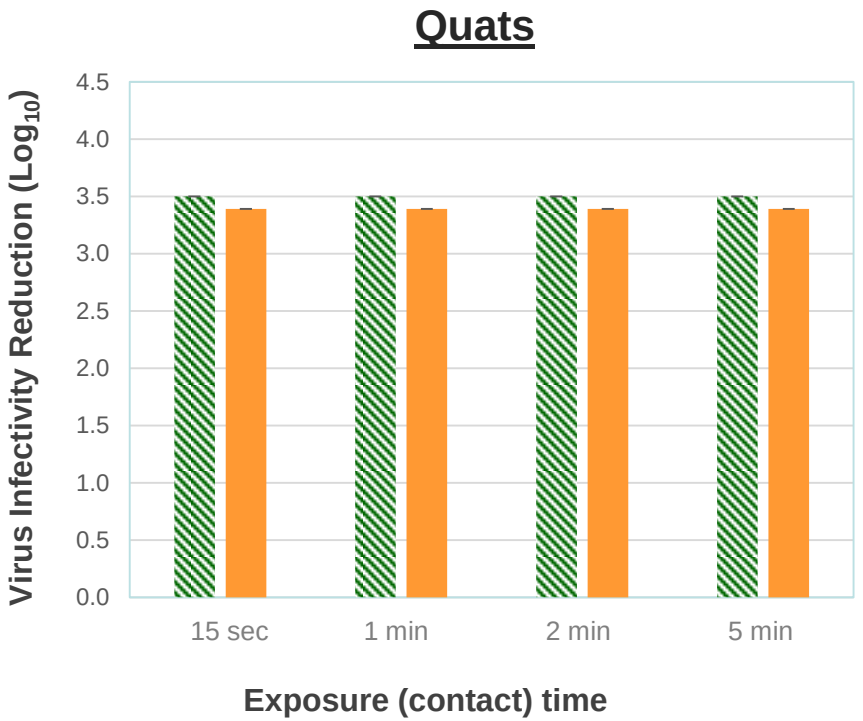
Efficacy of PAA on ZIKV is also Organic-dependent



* A complete inactivation of virus was achieved at 5% serum.

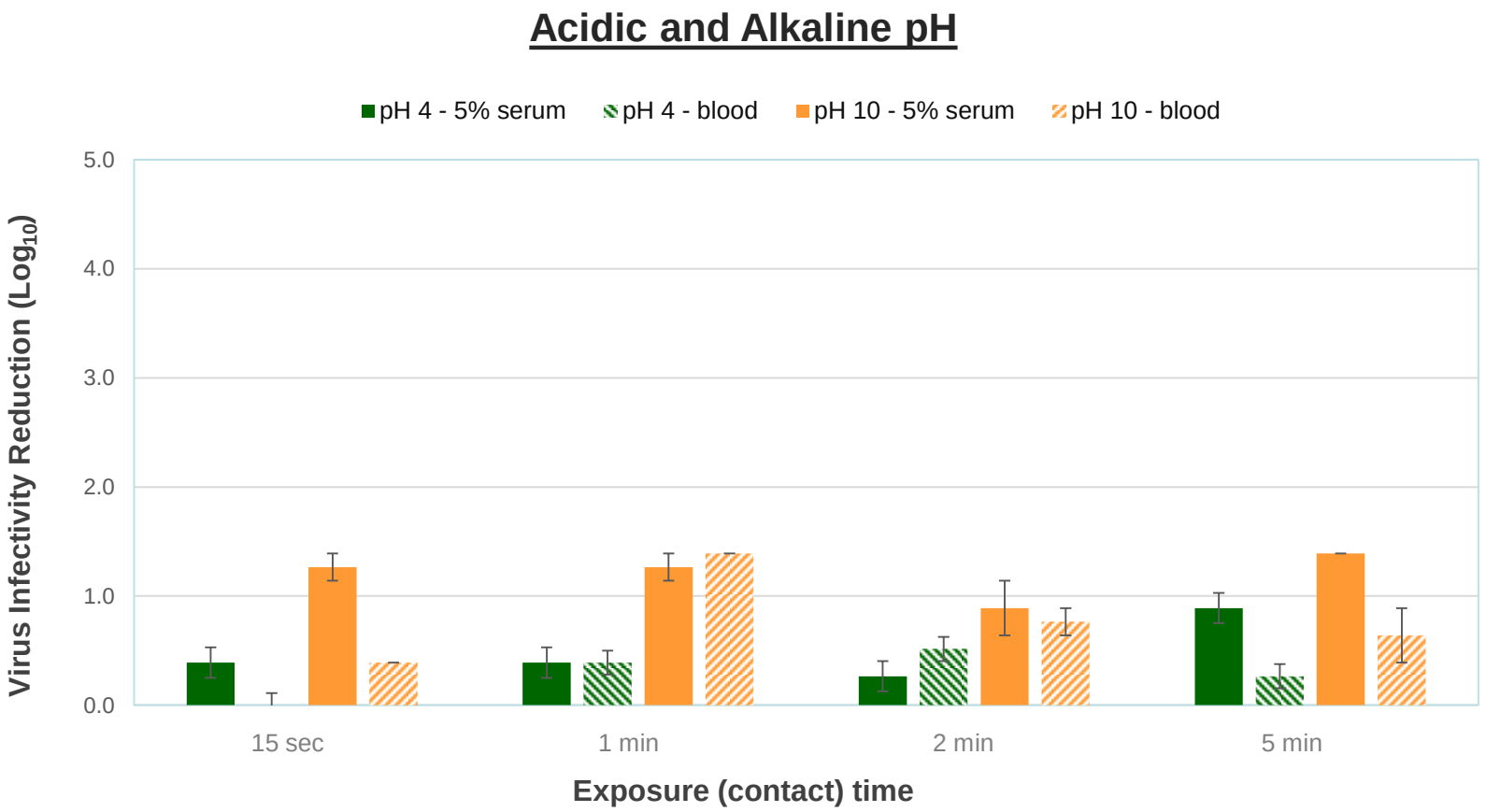
ZIKV is Sensitive to Alcohol and Quats w/ or w/o Blood





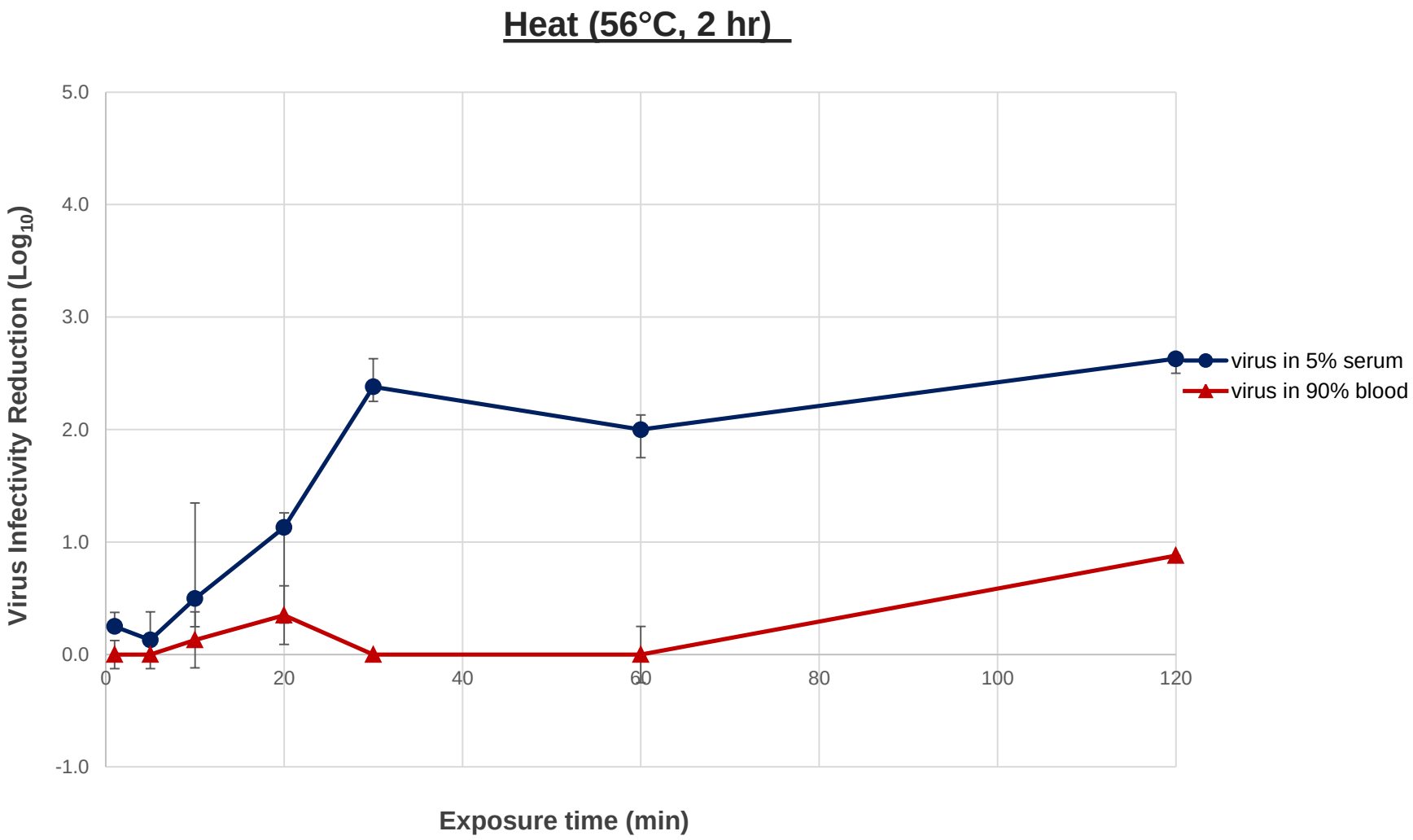
Note: A complete inactivation of virus was achieved in all cases.

ZIKV is Resistant to pH 4 and pH 10

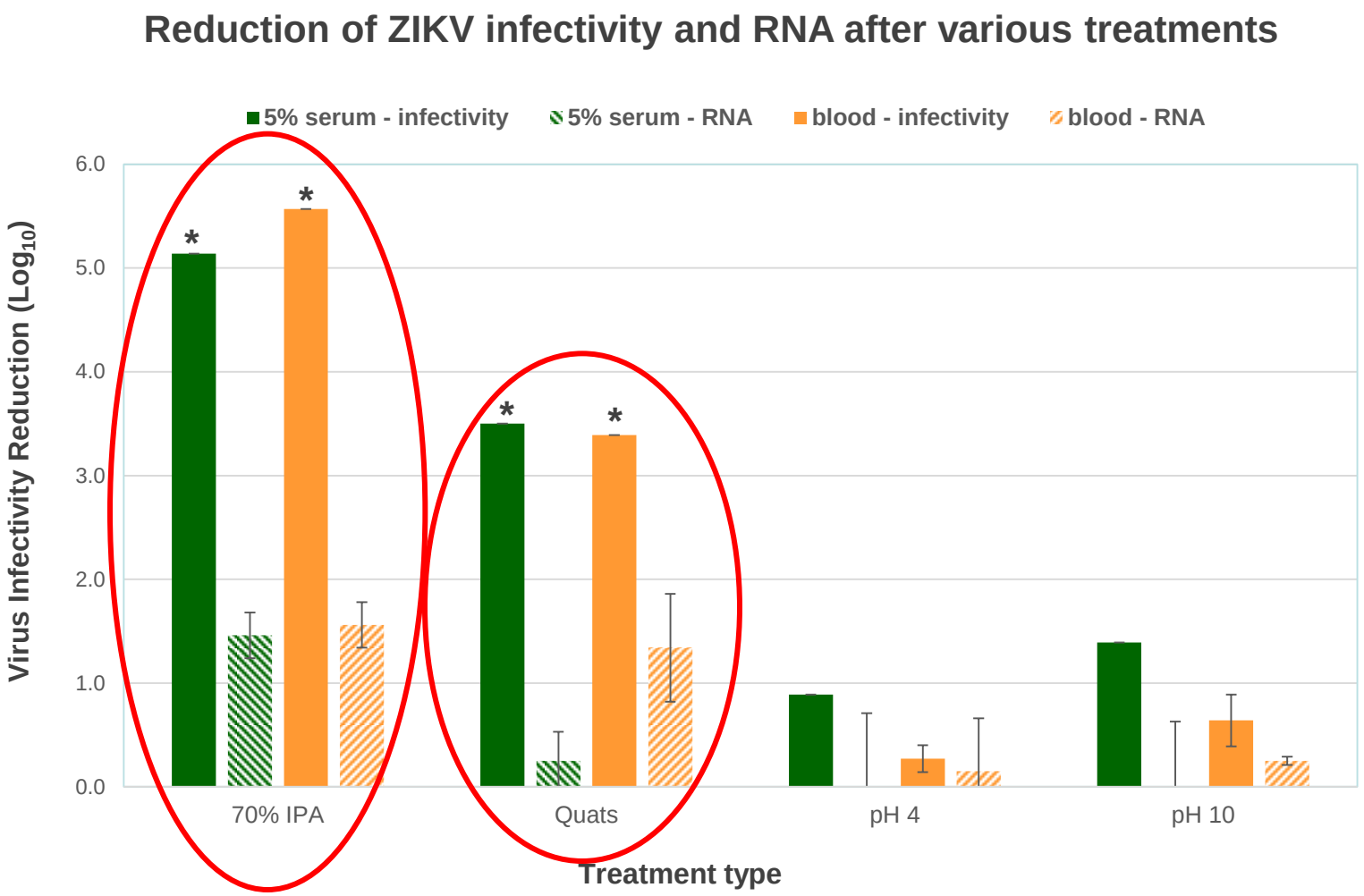


In general, an enteric flavivirus such as BVDV can be considered a worst case for low pH for env viruses, and a worst case for high pH for all viruses. Parvoviruses are not worst case.

Heat Inactivation of ZIKV

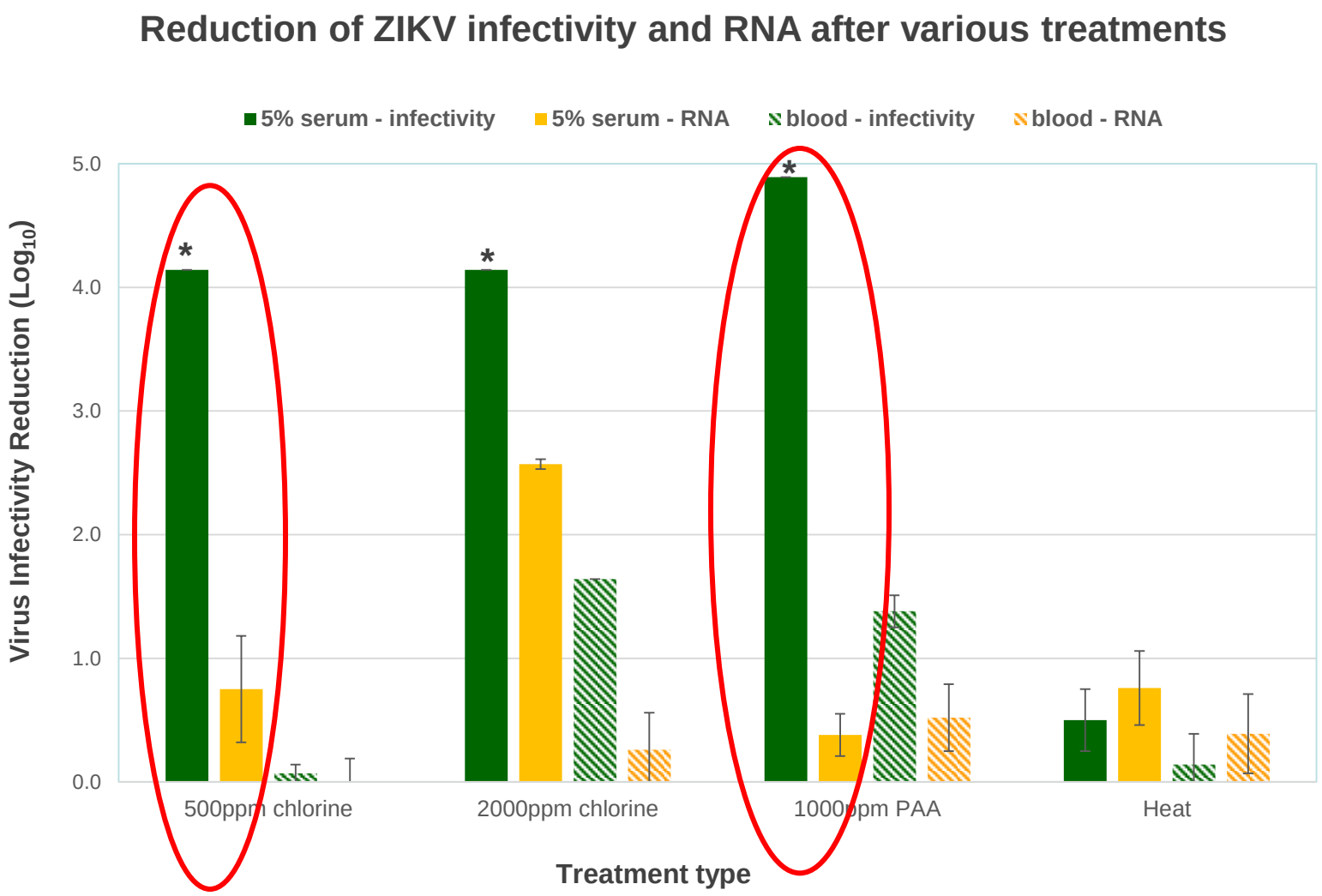


Infectivity loss vs. RNA damage



* A complete inactivation of virus was achieved in IPA and Quats under 5% serum and 90% blood.

Infectivity loss vs. RNA damage



* 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

Genus	Host	Example virus	Transmission
Flavivirus	Humans Mammals Mosquitos Ticks	Zika virus	mosquito-borne
		Dengue Fever virus	mosquito-borne
		Japanese Encephalitis virus	mosquito-borne
		St. Louis Encephalitis virus	mosquito-borne
		West Nile virus	mosquito-borne
		Yellow Fever virus*	mosquito-borne
Hepacivirus	Humans	Hepatitis C virus	sex, blood
Pegivirus	Mammals	GB virus A	unknown
Pestivirus	Mammals	Bovine Viral Diarrhea virus	Vertical (parental)

** first human virus discovered in 1901 by Walter Reed*

Comparison of Susceptibility of ZIKV, BVDV & WNV

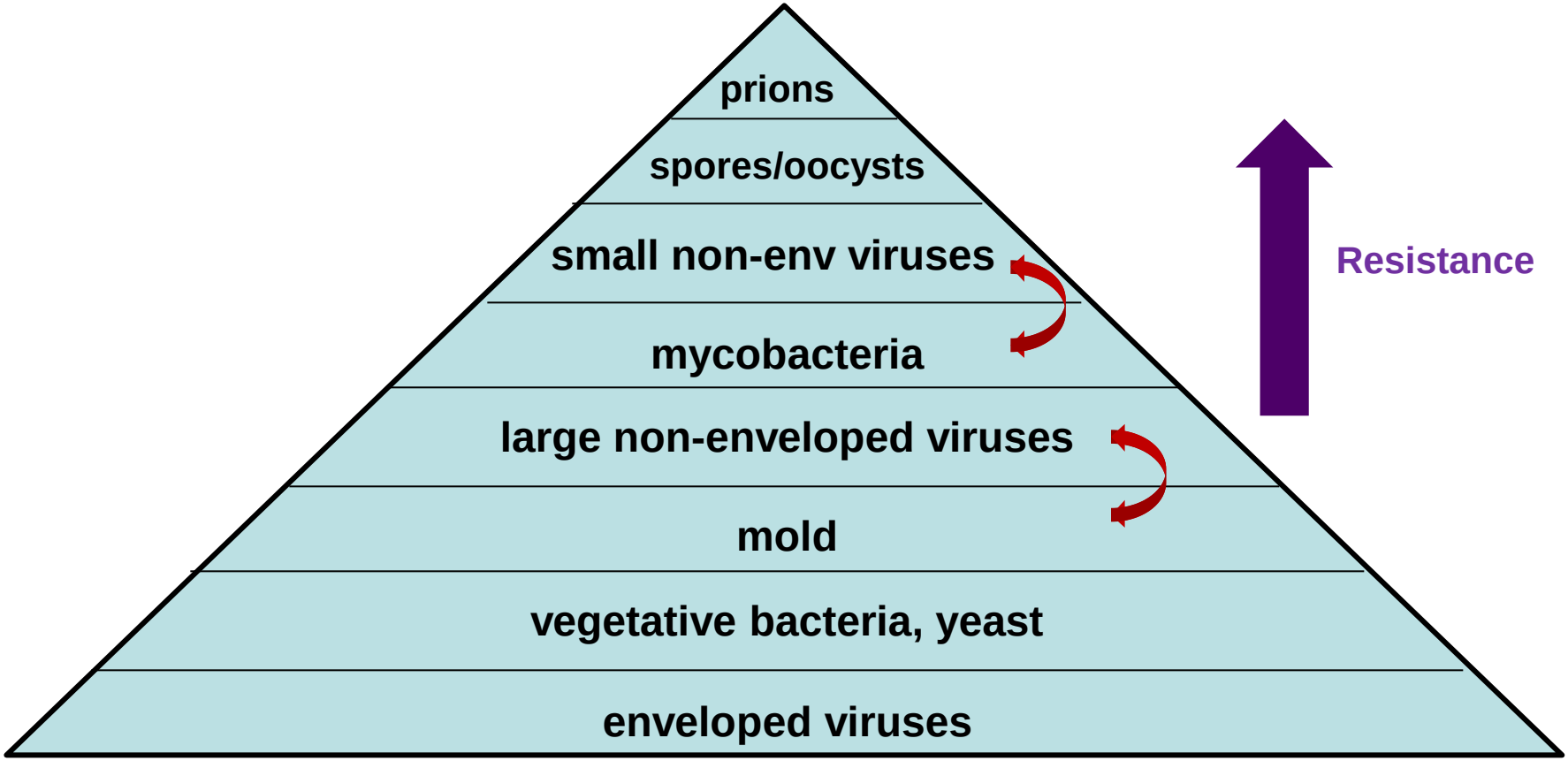
Treatment	Contact time	Log_{10} reduction in infectivity					
		ZIKV		BVDV		WNV	
		5% serum	90% blood	5% serum	90% blood	5% serum	90% blood
pH 4	5 min	0.9 ± 0.0	0.3 ± 0.1	1.1 ± 0.3	0.1 ± 0.0	1.4	0.3
pH 10	5 min	1.4 ± 0.0	0.6 ± 0.3	1.0 ± 0.3	0.1 ± 0.1	2.0	1.3
70% IPA	15 sec	≥ 5.1	≥ 5.6	≥ 4.3	≥ 3.3	≥ 3.5	≥ 2.6
Quat/Alcohol	15 sec	≥ 3.5	≥ 3.4	≥ 4.3	≥ 3.3	≥ 3.5	≥ 2.6
500 ppm chlorine	5 min	≥ 4.1	0.1 ± 0.1	3.7 ± 0.3	0.4 ± 0.0	≥ 3.5	≥ 2.6
2000 ppm chlorine	5 min	≥ 4.1	1.6 ± 0.0	4.0 ± 0.3	0.6 ± 0.1	≥ 3.5	≥ 2.6
1000 ppm PAA	5 min	≥ 4.9	1.4 ± 0.1	3.8 ± 0.0	1.2 ± 0.1	≥ 3.5	≥ 2.6
Heat (56°C)	20 min	1.3 ± 0.3	0.1 ± 0.3	1.0 ± 0.2	0.1 ± 0.1	2.5	1.1

≥ *Denotes complete inactivation of virus*

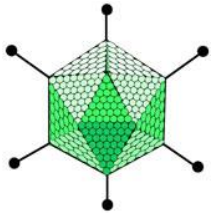
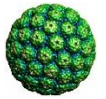
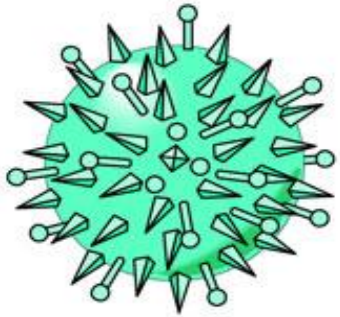
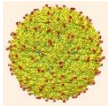
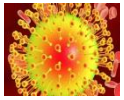
How Unique is ZIKV Compared to Other Viruses?

- ***How does ZIKV compare to non-flaviviruses?***

The “Hierarchy” of Micro-organism Resistance



Comparing ZIKV and Other Viruses

	Enveloped	Non-enveloped
DNA	 herpes simplex virus	 adenovirus  simian virus 40
RNA	 influenza virus  BVDV  ZIKV	 feline calicivirus (norovirus)  poliovirus

Comparing ZIKV and Other Viruses for Heat Resistance

Virus:

- BVDV
- HSV-1
- Influenza
- ZIKV
- Adenovirus 5
- FCV
- Poliovirus
- SV40

Heat condition:

- 46C
- 56C
- 65C
- 5 min
- 20 min
- 60 min
- 2 hr
- 3 hr

Matrix / Carrier:

- Glass surface
- Stainless steel
- Liquid - 5% serum
- Liquid - 100% serum

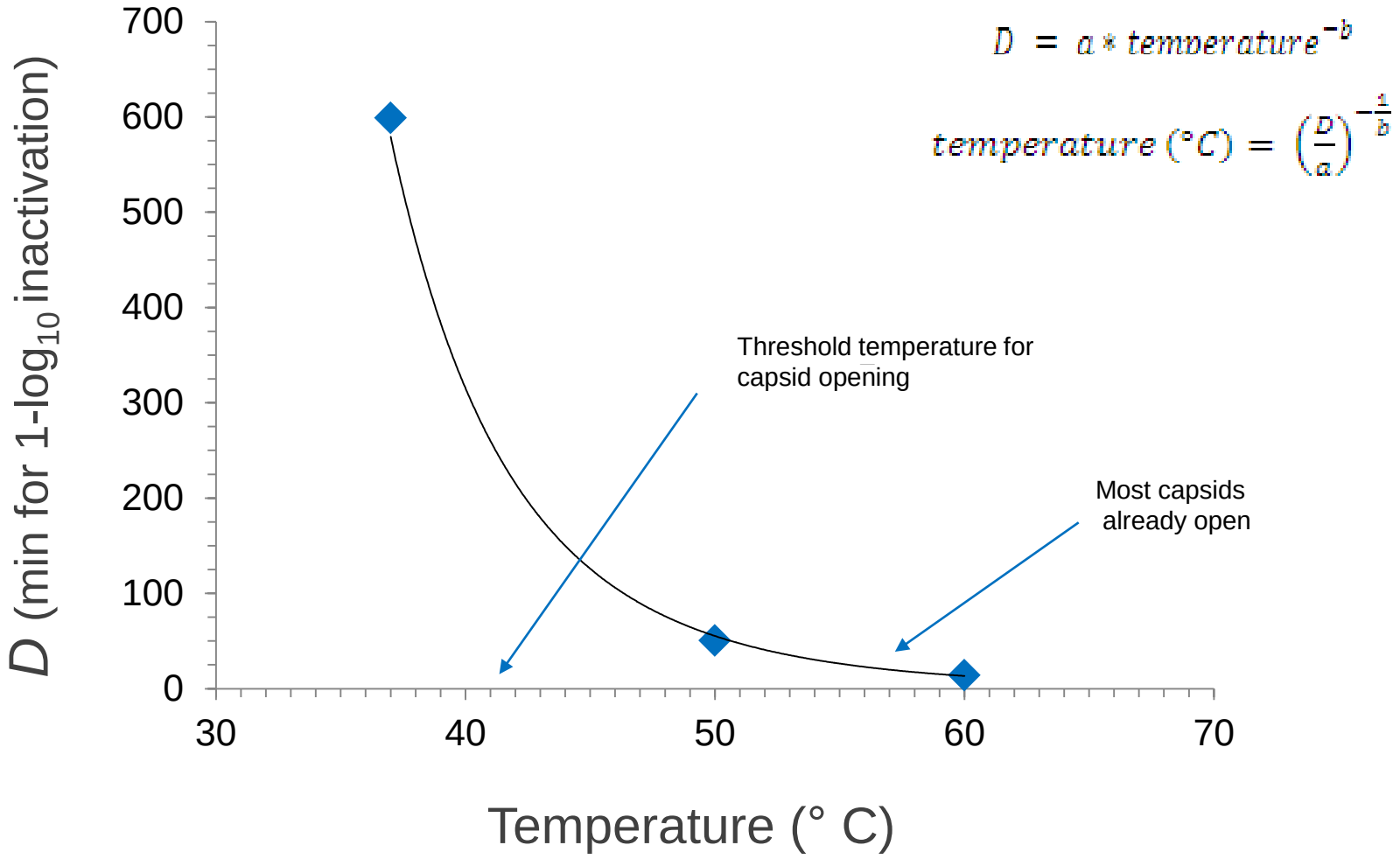
Heat Inactivation of ZIKV on Surface vs. in Liquid

Temp.	Contact time	Log_{10} reduction in infectivity*			
		Glass	Steel	Medium	Serum
46°C	5 min	0.7	0.0	0.0	0.0
	20 min	1.4	0.4	0.3	0.0
	60 min	1.7	1.0	0.6	0.1
56°C	5 min	0.5	0.7	0.0	0.1
	20 min	1.3	1.8	0.5	0.5
	60 min	2.0	2.6	1.7	2.2
65°C	5 min	0.7	0.5	0.0	0.0
	20 min	2.8	3.4	3.8	3.9
	60 min	3.7	4.1	≥ 4.3	≥ 4.2

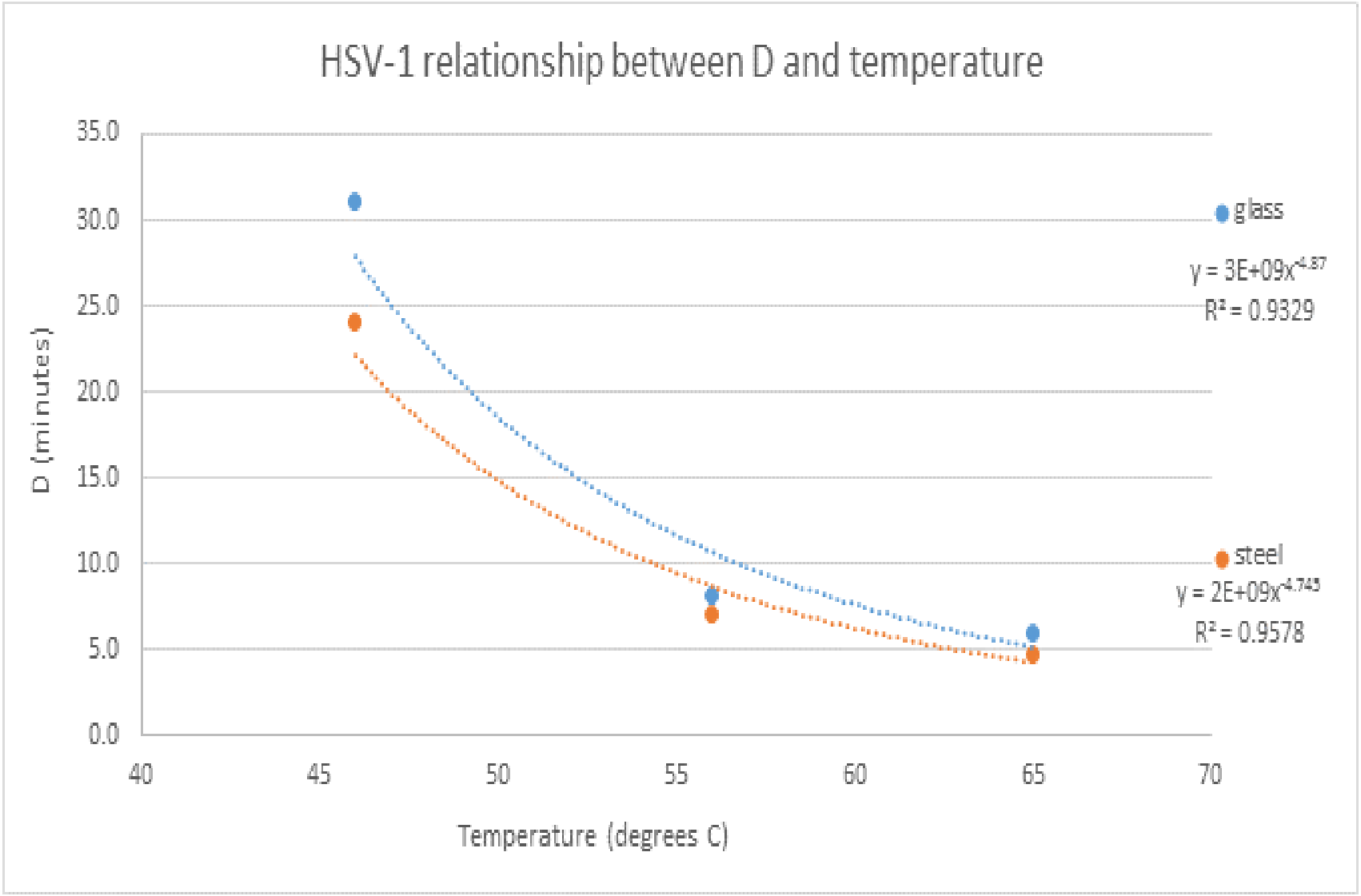
≥ Denotes complete inactivation of virus

** Results represent the average from two independent experiments*

Heat Inactivation of Virus: D Value



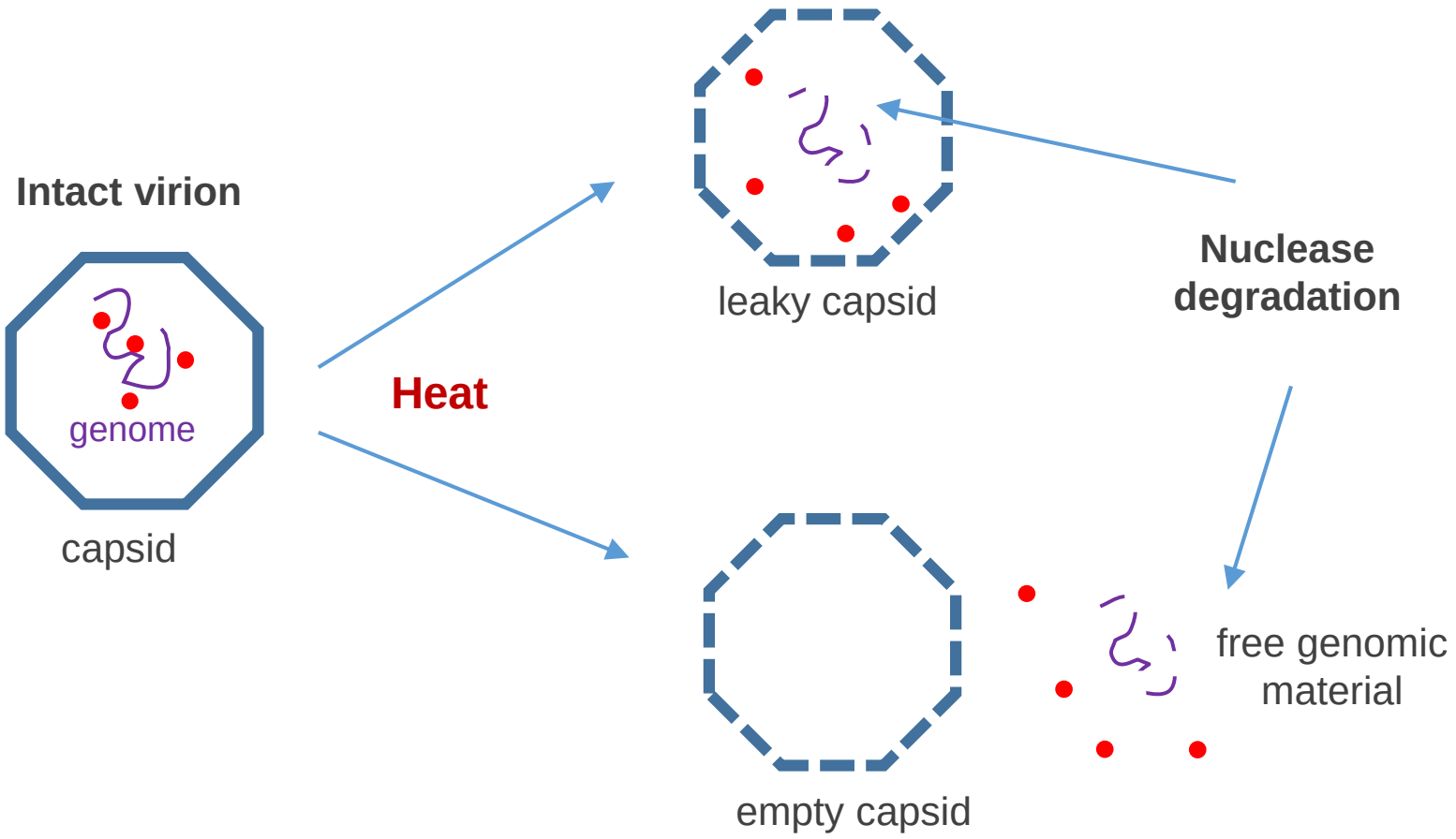
Relationship between D and temperature



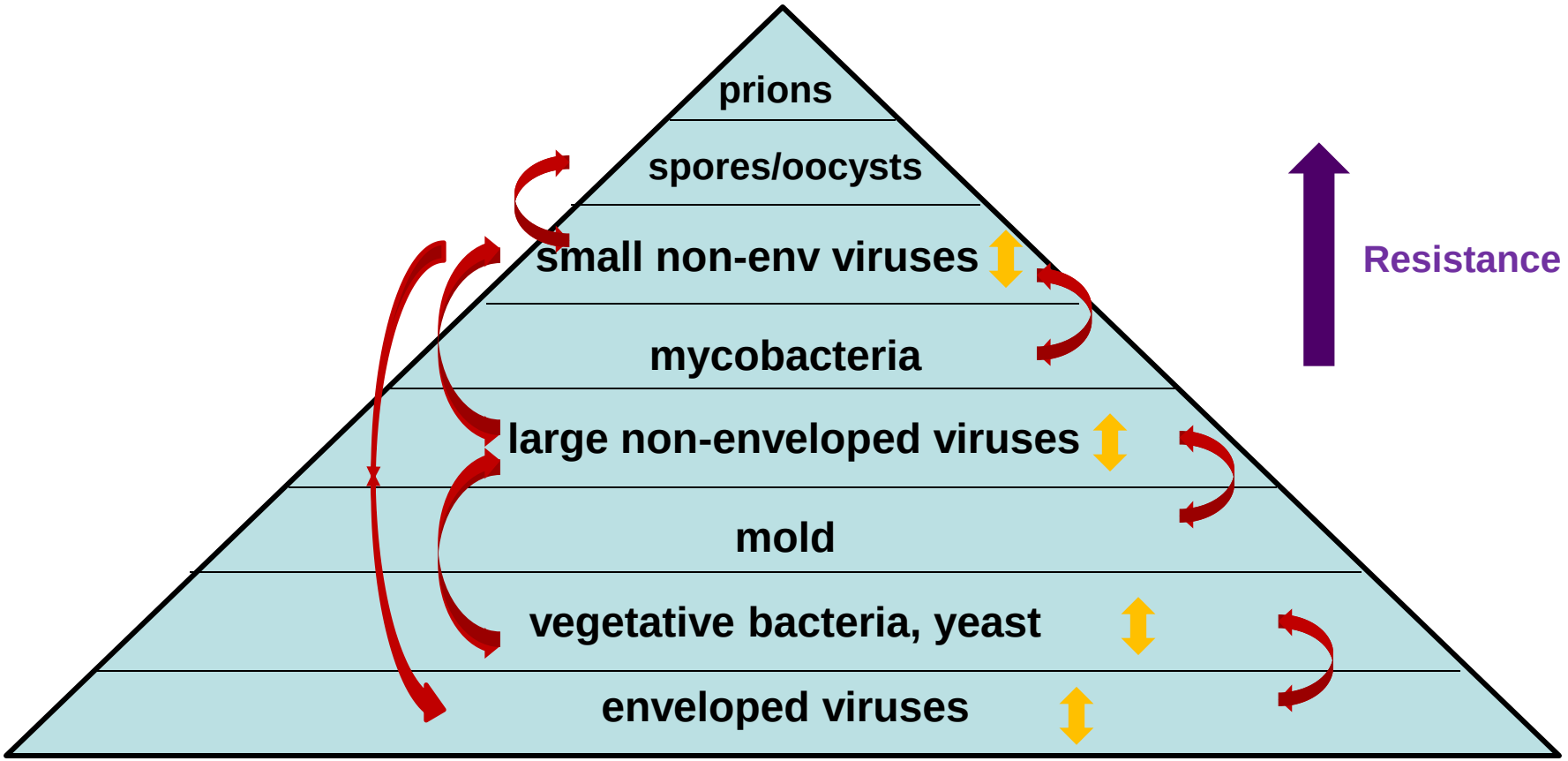
Viruses Exhibit Different Resistance to Heat

Virus	Env	Temp.	D (min for 1-log_{10} inactivation)				
			Glass	Steel	Medium	Serum	Ave.
HSV-1	Y	65°C	6.0	4.8	4.8	4.2	5.0
PV-1	N	65°C	4.0	4.4	3.9	9.3	5.4
AD5	N	65°C	u.d.	6.0	5.6	5.1	5.6
ZIKV	Y	65°C	7.2	6.0	5.5	5.3	6.0
FCV	N	65°C	6.7	6.7	6.5	6.3	6.6
FLU	Y	65°C	13.2	9.4	9.6	9.5	10.4
BVDV	Y	65°C	7.5	8.1	13.1	15.0	10.9
SV40	N	65°C	19.8	18.9	12.7	14.4	16.5

Proposed Mechanism of Heat Inactivation on Virus



The “Hierarchy” is Not Always True



Summary

- 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

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