

# Virucidal activity of 70% Ethanol vs Enveloped and Non-Enveloped Viruses

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## OBJECTIVE:

Viruses contain genetic material packaged in a protein coat called the capsid. For some viruses, the capsid is surrounded by an envelope composed of a lipid bilayer derived from the host cell membrane (Figure 2). Virus structure determines the stability characteristics of the virus particle, such as resistance to chemical or physical inactivation (Lucas, 2010).

To maintain a safe environment at the Institute for Antiviral Research, it is important to understand which viruses are resistant to which chemicals. Thus, the objective of this experiment was to test survival of the viruses listed in table 1, when exposed to various chemicals. Neutral red dye is routinely used in antiviral assays and 70% ethanol is common disinfectant used. MEM & H<sub>2</sub>O were used as negative controls. Mt. Dew was also tested because we thought it would be fun.

Enveloped	Naked
Influenza A	Adenovirus-5
Zika virus	Enterovirus-71
	Enterovirus-68
	Rhinovirus-14

Table 1. Viruses tested

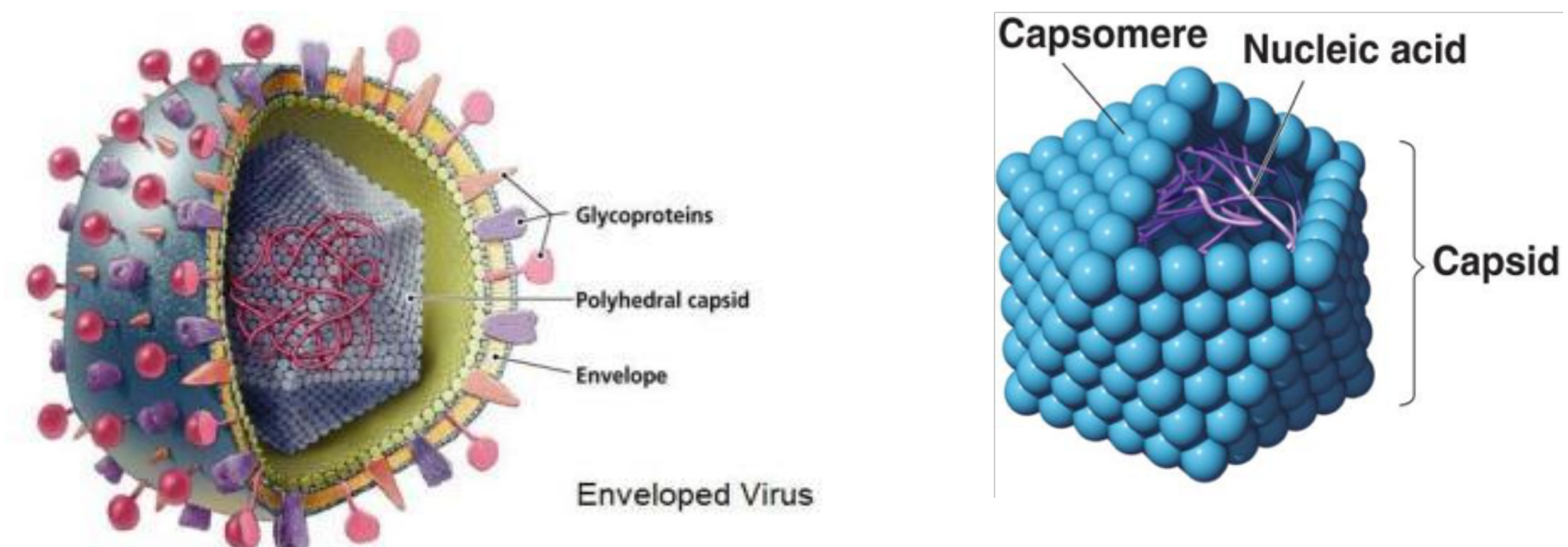


Figure 2. 3D model of (a) an enveloped virus and (b) a non-enveloped virus.

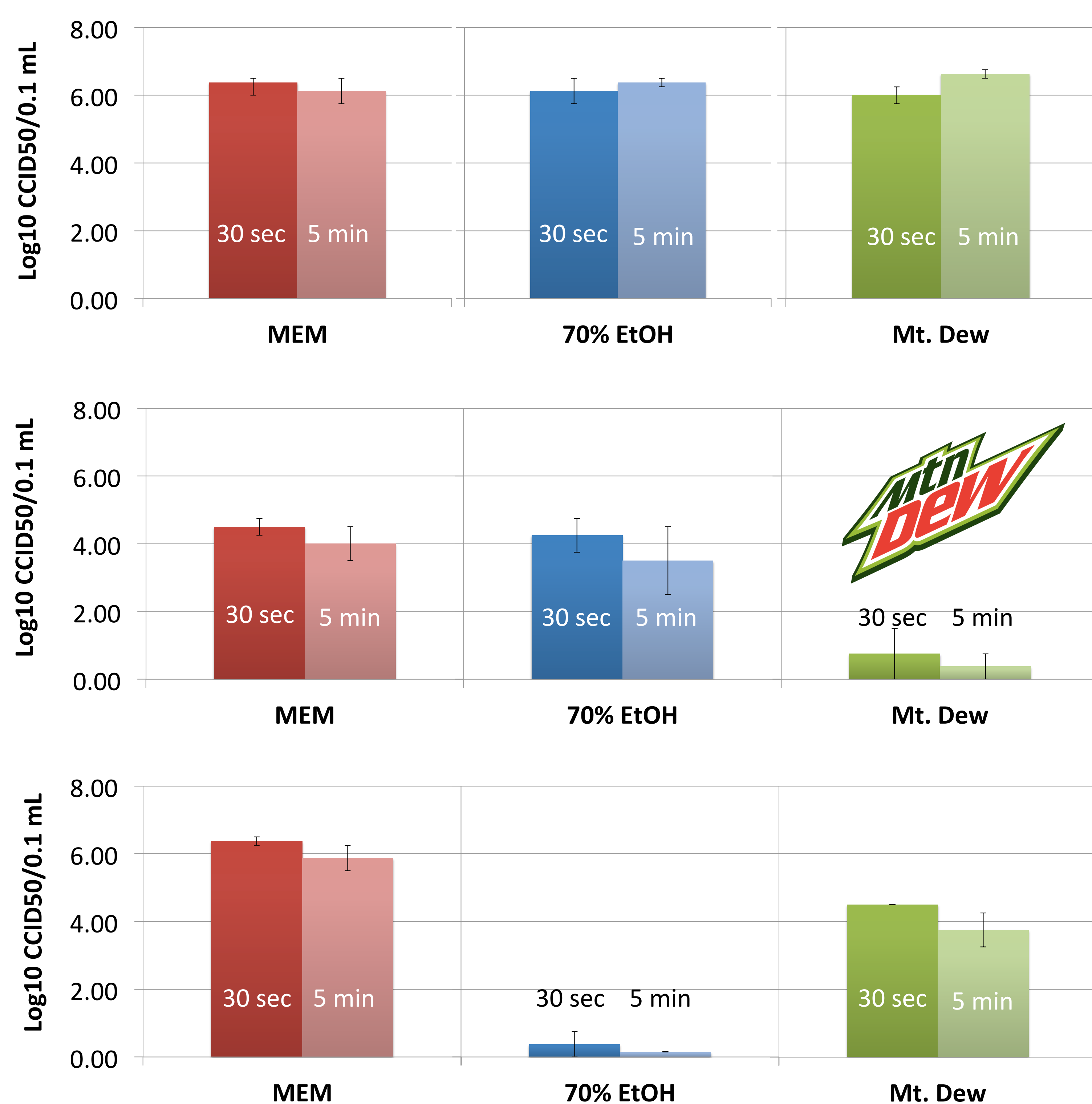


Figure 2. Surviving Enterovirus-71, Rhinovirus-14, and Zika virus after exposure in MEM (control), 70% ethanol, Mt. Dew for 30 sec and 5 min.

## MATERIALS AND METHODS:

Each virus was mixed in solution (10  $\mu$ L into 90  $\mu$ L) with 70% ethanol (diluted to 63% after virus was added), Mt. Dew, water, MEM (control) or 0.11% neutral red for 30 seconds, 5 minutes, and 2 hours. Viral titers of the samples were determined using a standard endpoint dilution assay in triplicate wells of 96-well plates containing Vero 76 cells and incubated at 37°C and 5% CO<sub>2</sub>. Viral cytopathic effect was visually read and recorded on day 6. The CCID<sub>50</sub> for each sample was calculated using the Reed-Muench equation (1948).

COMPOUND	Virus	30 SEC.	5 MIN.	2 HR.
0.33% Neutral Red Dye	Adenovirus (naked)	0.38	-0.13	0.50
70% Ethanol		1.68	1.25	6.50*
H2O		-0.38	-0.13	0.13
Mt. Dew Voltage		0.00	-0.13	nt
0.33% Neutral Red Dye	Enterovirus 68 (naked)	-0.13	0.13	0.00
70% Ethanol		2.38*	1.88	4.25**
H2O		0.25	0.00	0.13
Mt. Dew Voltage		0.50	1.13*	nt
0.33% Neutral Red Dye	Enterovirus 71 (naked)	0.00	-0.13	-0.75
70% Ethanol		0.25	-0.25	3.63*
H2O		0.38	0.00	0.25
Mt. Dew Voltage		0.38	-0.50	nt
0.33% Neutral Red Dye	Influenza A (enveloped)	0.38	0.25	1.00
70% Ethanol		4.84***	NT	5.50**
H2O		-0.25	0.00	0.00
Mt. Dew Voltage		2.75	4.13	nt
0.33% Neutral Red Dye	Rhinovirus (naked)	0.25	-0.13	-0.25
70% Ethanol		0.25	0.50	3.88*
H2O		0.38	0.00	-0.25
Mt. Dew Voltage		3.75*	3.63*	nt
0.33% Neutral Red Dye	Zika virus (enveloped)	0.50	0.00	0.88
70% Ethanol		6.00**	5.88**	5.75***
H2O		0.13	0.38	0.13
Mt. Dew Voltage		1.88**	2.13	nt

Table 2: Log reduction value compared with untreated control (MEM), e.g, 1.0 log = 90% reduction, 2.0 = 99% reduction, etc. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 by students t-test compared with MEM control.

## CONCLUSIONS/RESULTS:

Despite their relative simplicity, virus structures vary significantly. These variations affect the virus's susceptibility to certain compounds. For example, the data show that Mt. Dew kills Rhinovirus-14 more efficiently than 70% ethanol. This could be due to the acidity of Mt. Dew, which has a pH of 3.22 (Shelton). The results also show that some non-enveloped viruses are moderately resistant to 70% ethanol such as Rhinovirus-14, while others are highly resistant such as Enterovirus-71. Based on the data we can conclude that enveloped viruses are extremely vulnerable to 70% ethanol. These findings should be taken into consideration when working with active virus in laboratory or healthcare settings.

## Citations:

Lucas, W. 2010. Viral Capsids and Envelopes: Structure and Function. LS Shelton, Robert B., DDS MAGD. "PH Values Of Common Drinks." *Dentist in Longview Texas*. Web. 06 Apr. 2017.