RD83582201-0

ENABLING ADAPTIVE UV AND SOLAR-BASED DISINFECTION SYSTEMS TO REDUCE THE PERSISTENCE OF VIRAL PATHOGENS IN WASTEWATER FOR SUSTAINABLE REUSE

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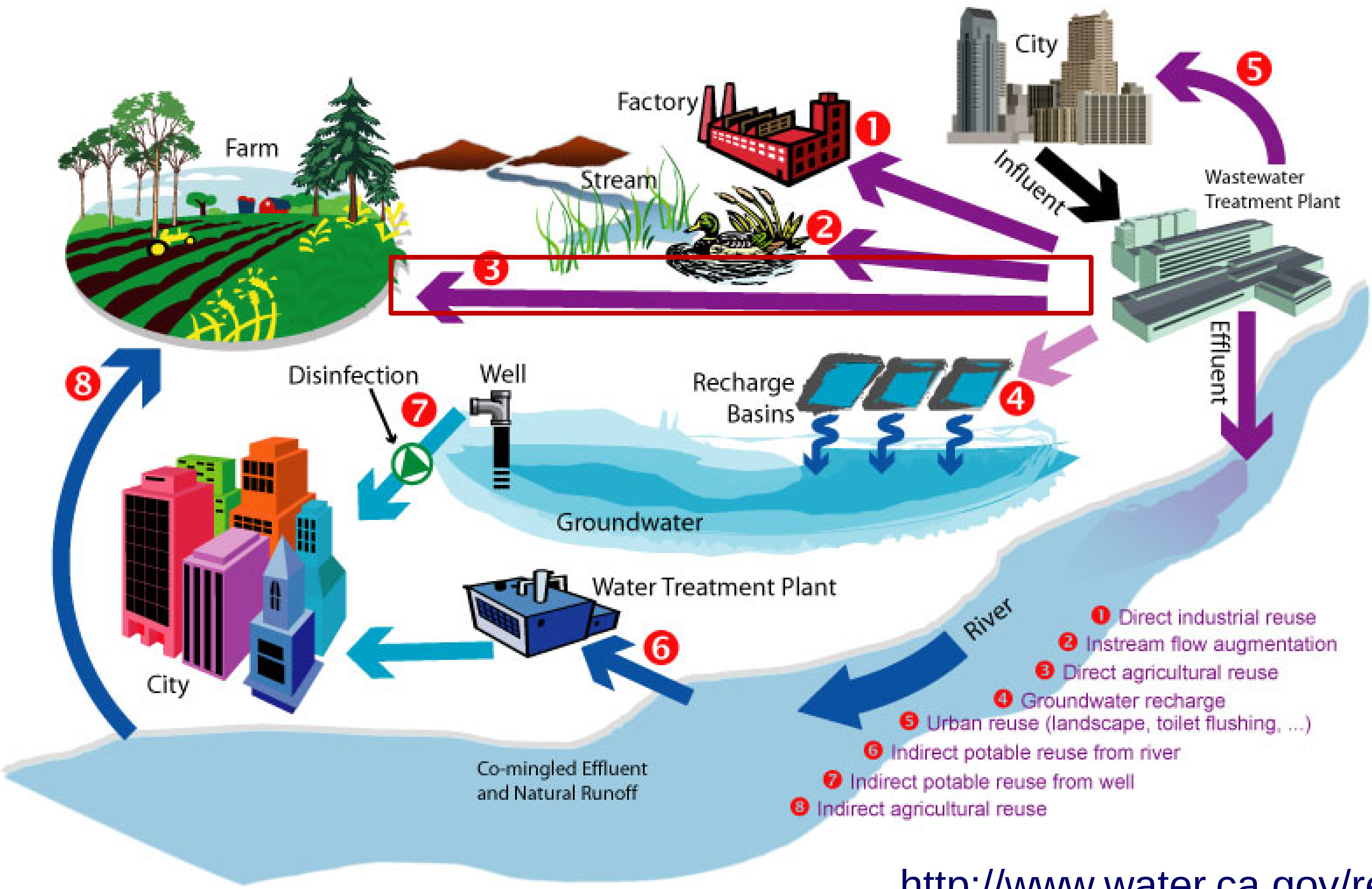
University of Illinois

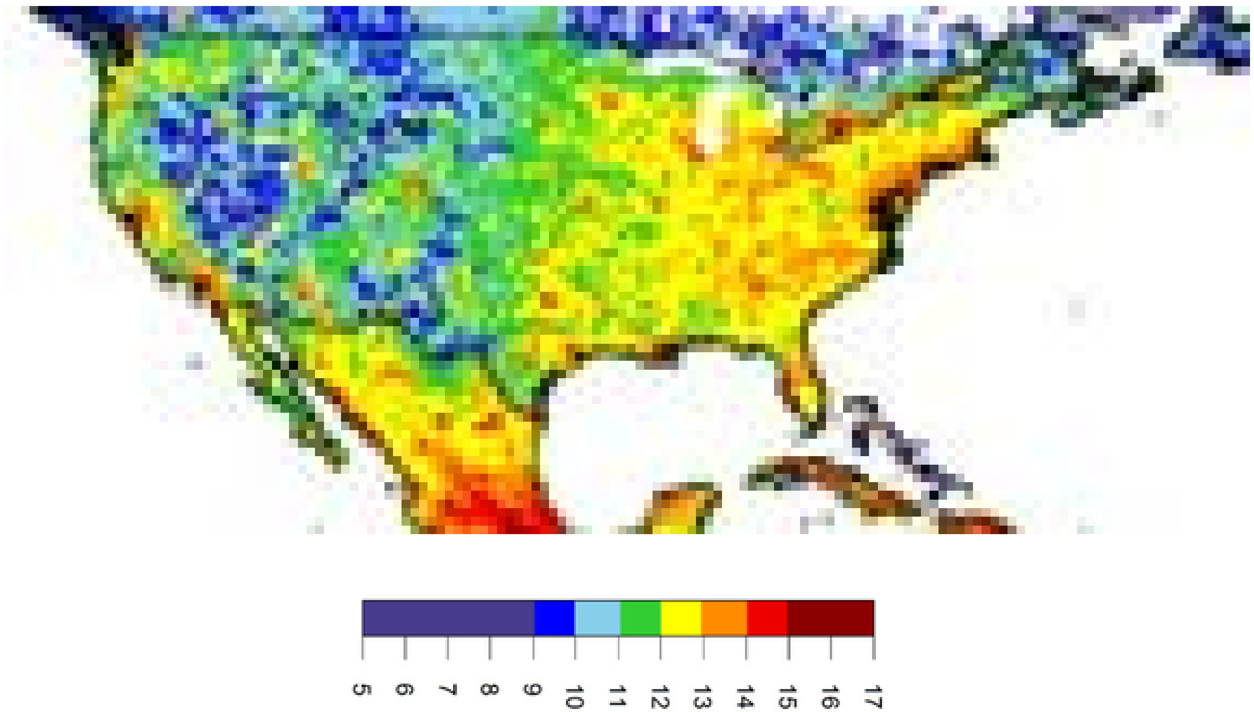
Transport and Survival of Pathogens in Aquatic Environment: Water Reuse Application



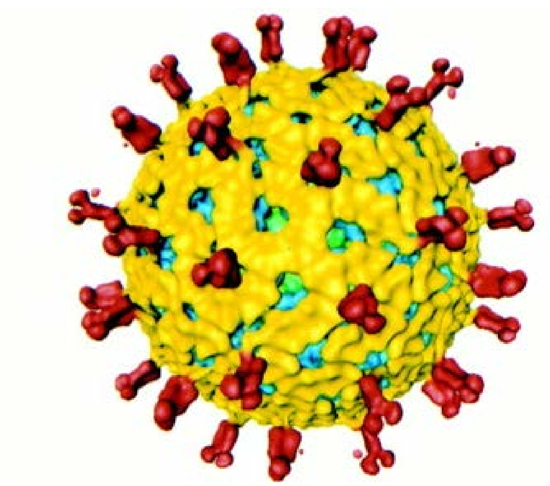
http://www.water.ca.gov/recycling/

Rotavirus: One of the Most Common Diarrhea Causing Viruses

Occurrence and Emission of Rotavirus to Surface Waters



Rotavirus emissions (log10 viral particles / grid / year)



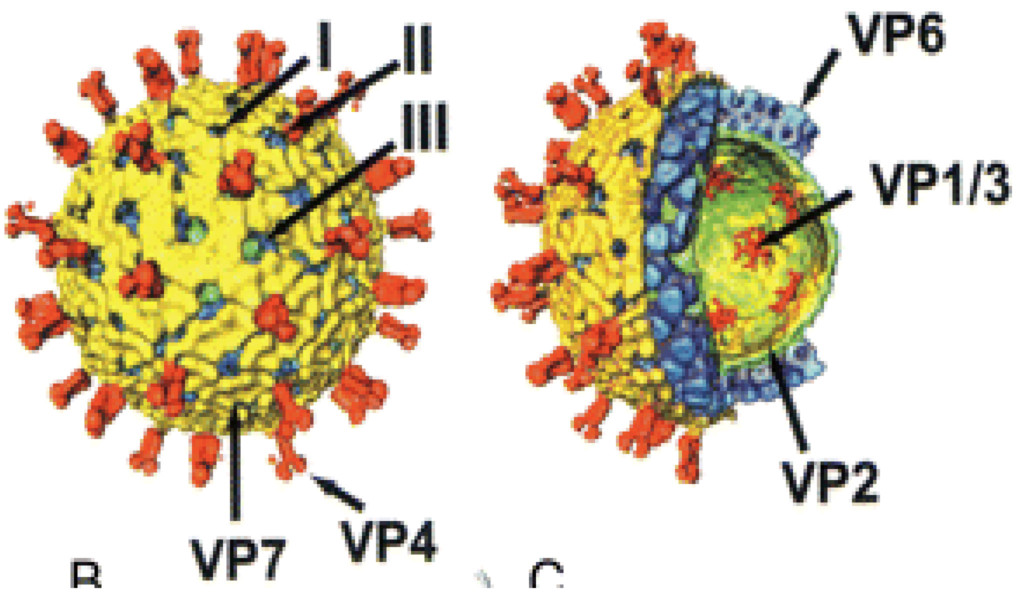
Rotavirus, Pesavento et al., 2001)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC29265/figure/F2/

Kiulia et al., Pathogens. 2015 May 13;4(2):229-55.

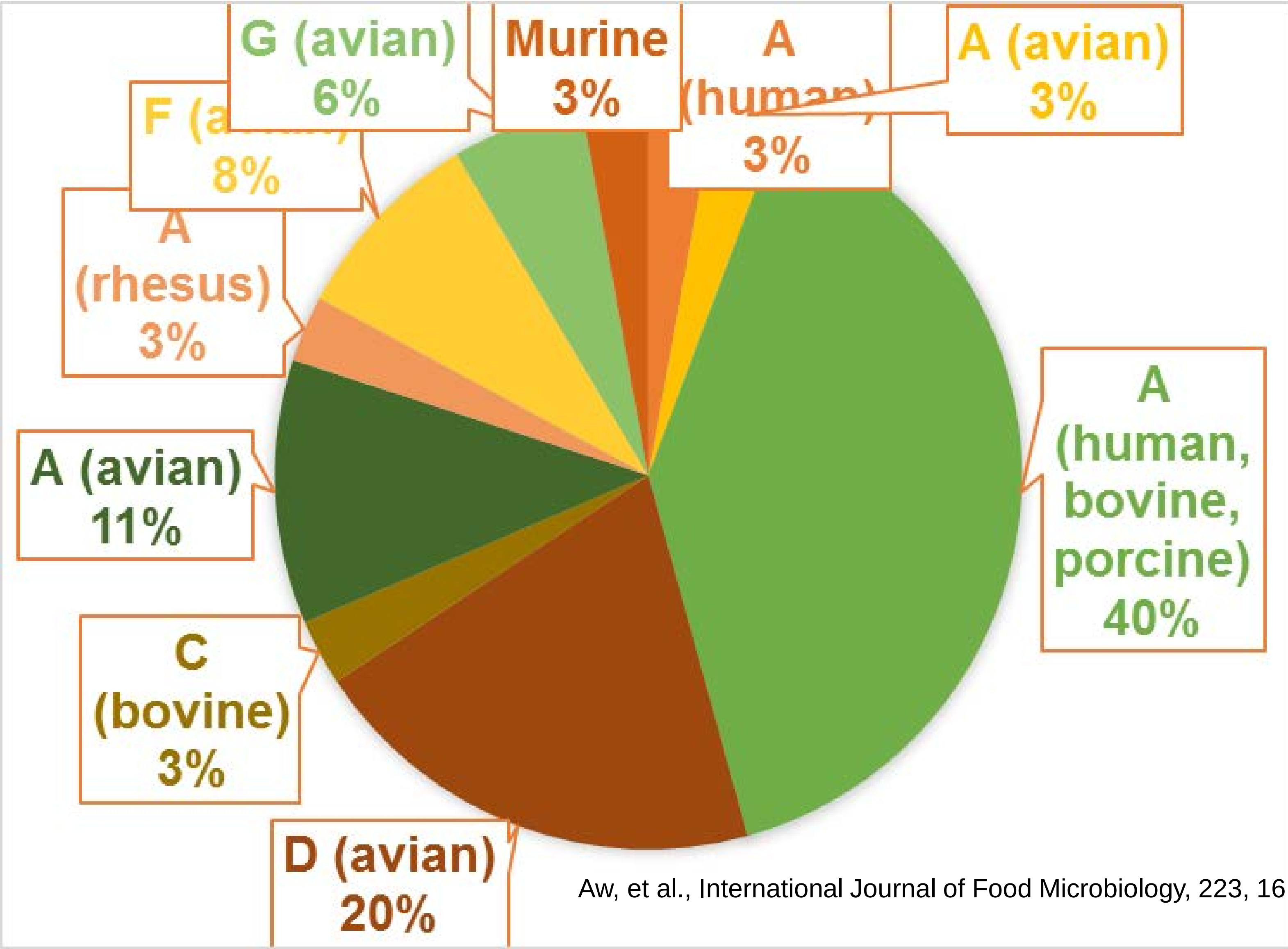
Rotavirus Serotypes

- **The capsid consists of VP2, VP6, and VP7**
- VP6: 7 serological groups (A to G)
- VP7 (glycoprotein): 14 G serotypes
- VP4 (hemagglutinin protein): P serotypes



Desselberger, et al.(2009) Rotavirus and rotavirus vaccines.
British Med Bull (2009) 90 (1)

Human and Animal Rotavirus Found in Lettuces from AZ



Aw, et al., International Journal of Food Microbiology, 223, 16 April 2016

Reuse of Treated Wastewater

Urbana-Champaign Wastewater Treatment Facility

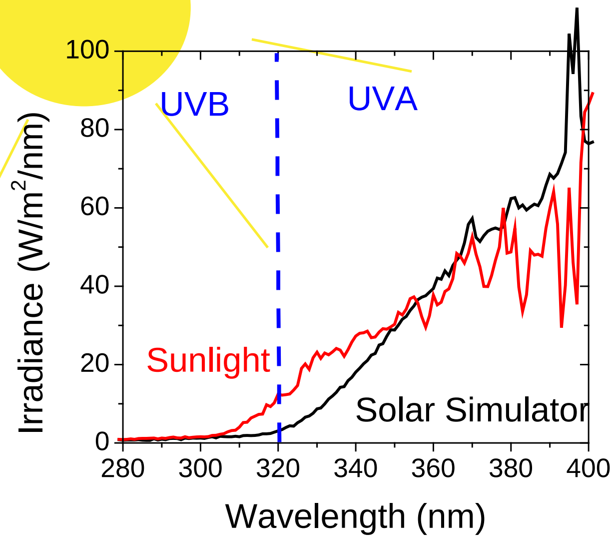
Cortland Reclamation for Irrigation

Project Objectives

1. Determine the molecular mechanisms responsible for virus inactivation
2. Determine factors required for effective virus inactivation by natural sunlight and UVC
3. Develop pond and UVC design guidelines to achieve reliable virus inactivation and elucidate trade-offs across and within dimensions of sustainability.

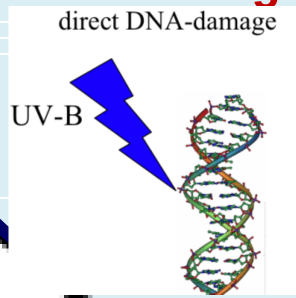
Sunlight Is The Most Important Factor Causing Disinfection In Surface Water

Sunlight



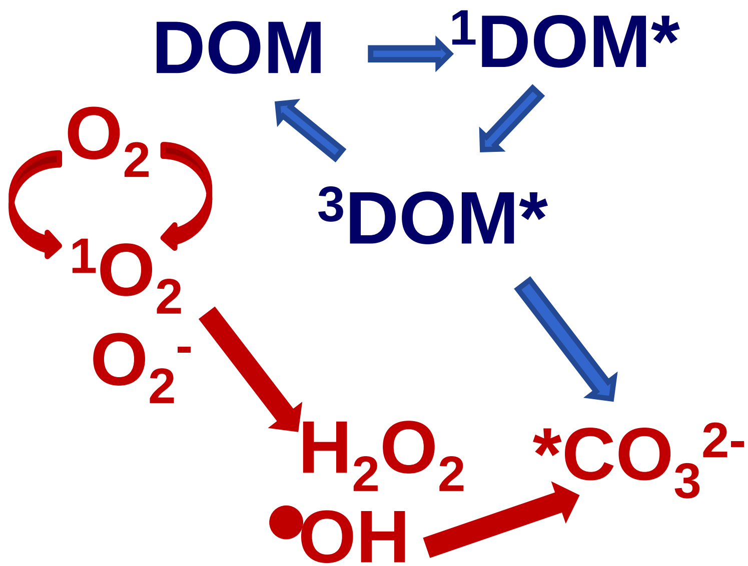
2006; Davies-Colley *et al*. 1997, 1999; Da Silva, A.K, et al., 2008;

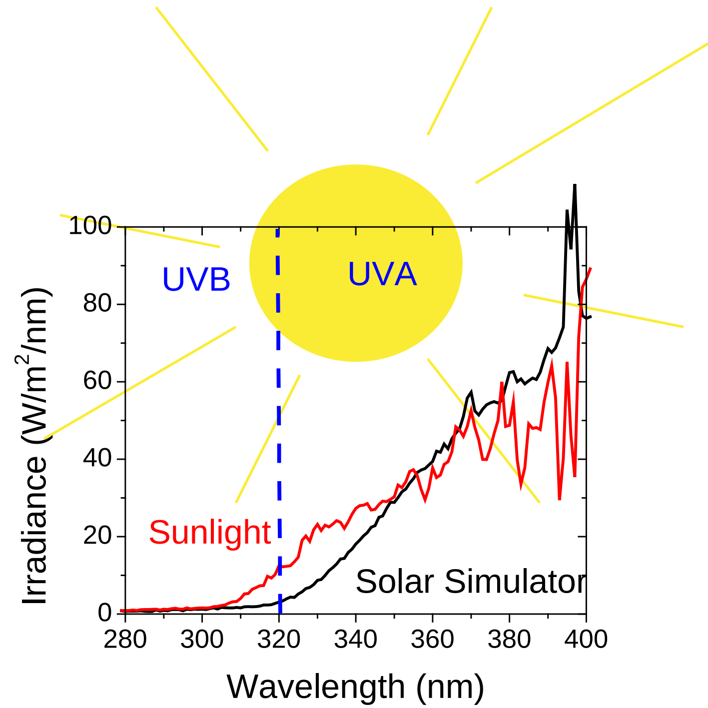
1. Direct UVB Damage

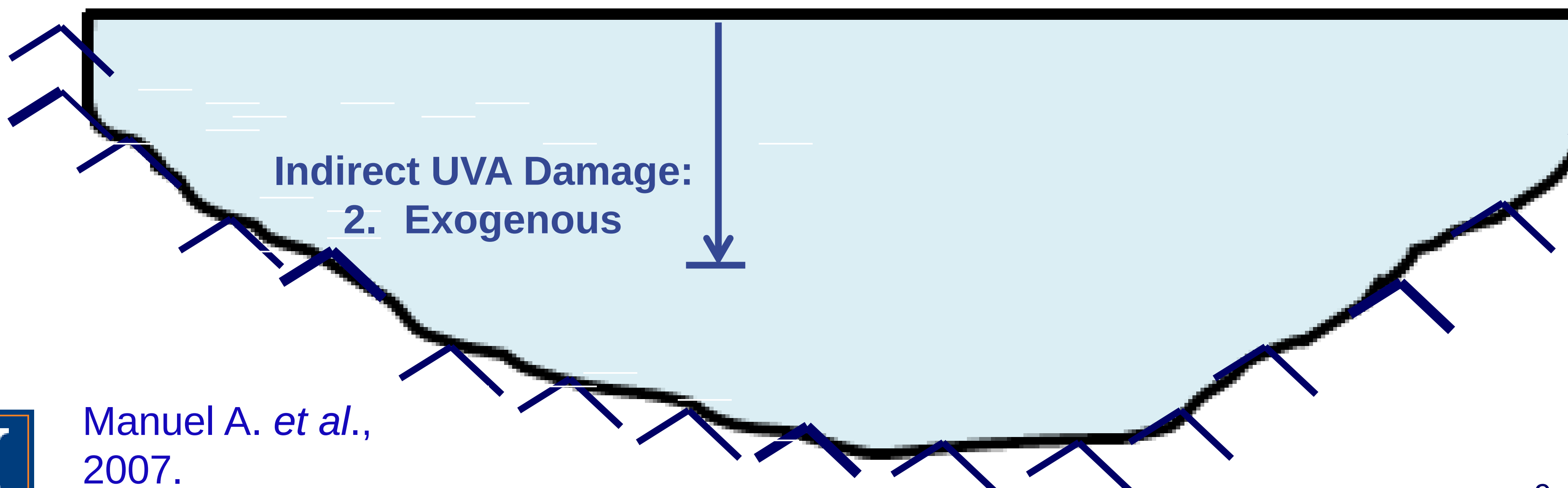


Causes direct effects on DNA by inducing dimerization of pyrimidine bases, leading to the formation of photo-products that may block DNA replication

http://www.thewatertreatments.com/waste-water-treatment-filtration-purify-sepration-sewage



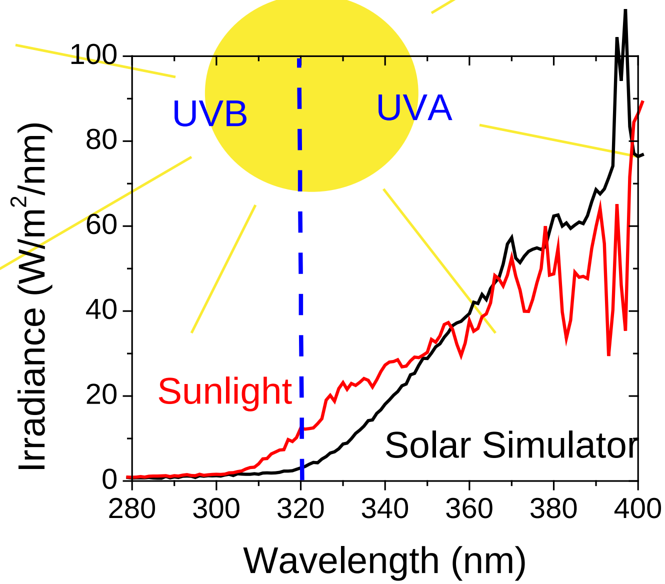




Manuel A. *et al.*, 2007.

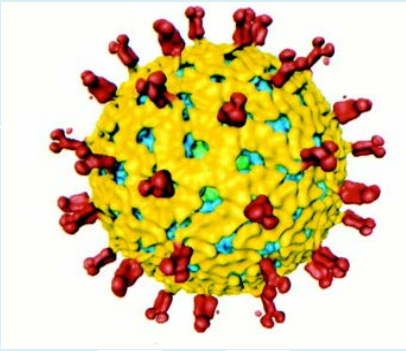
http://www.thewatertreatments.com/waste-water-treatment-filtration-purify-sepration-sewage

Indirect photo-oxidation damage is caused by generating ROS that damage the genome, proteins, and/or lipids. ROS formation is catalyzed by endogenous sensitizers.



Indirect UVA Damage:

2. Exogenous
3. Endogenous



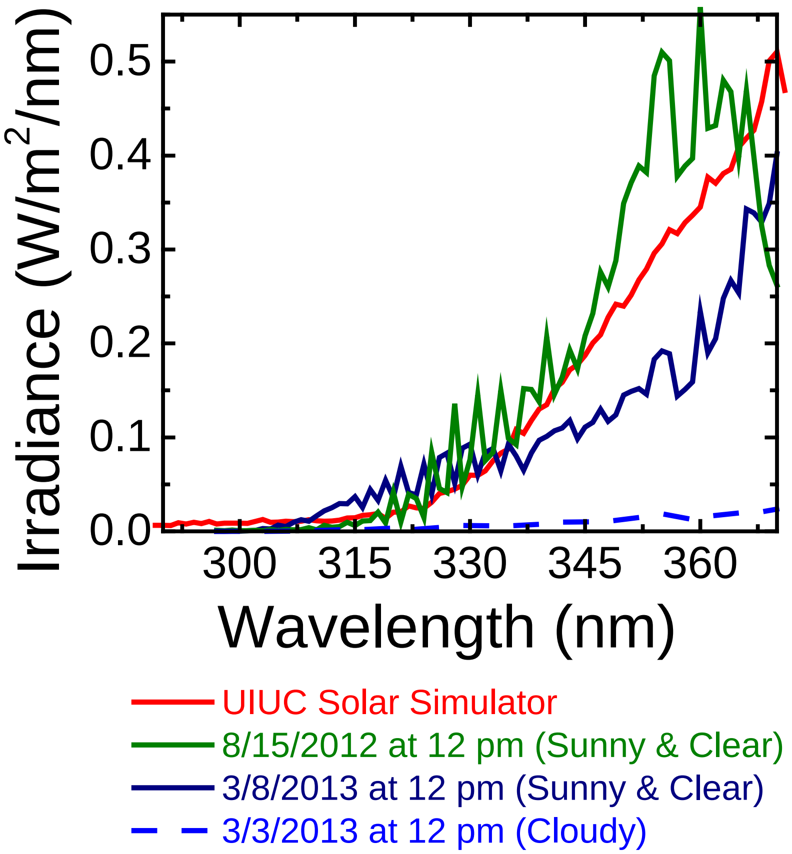
, Pesavento et al., 2001)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC 29265/figure/F2/

http://www.thewatertreatments.com/waste-water-treatment-filtration-purify-sepration-sewage

Experimental Setup

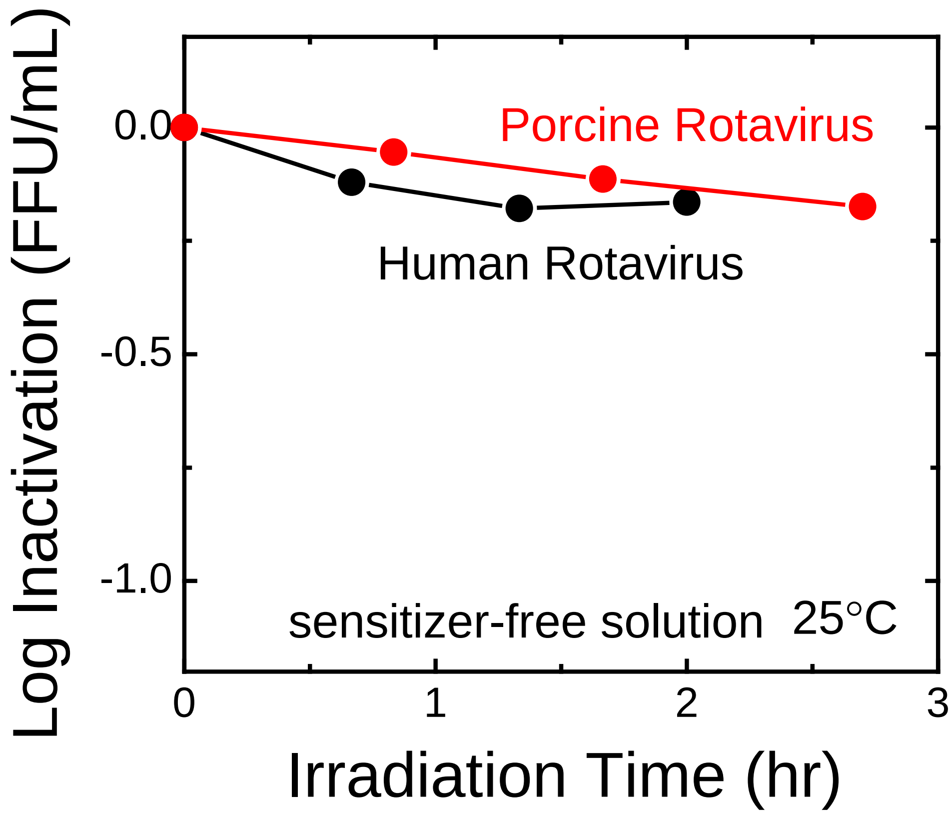
Xenon arc lamp with filters



Hypotheses

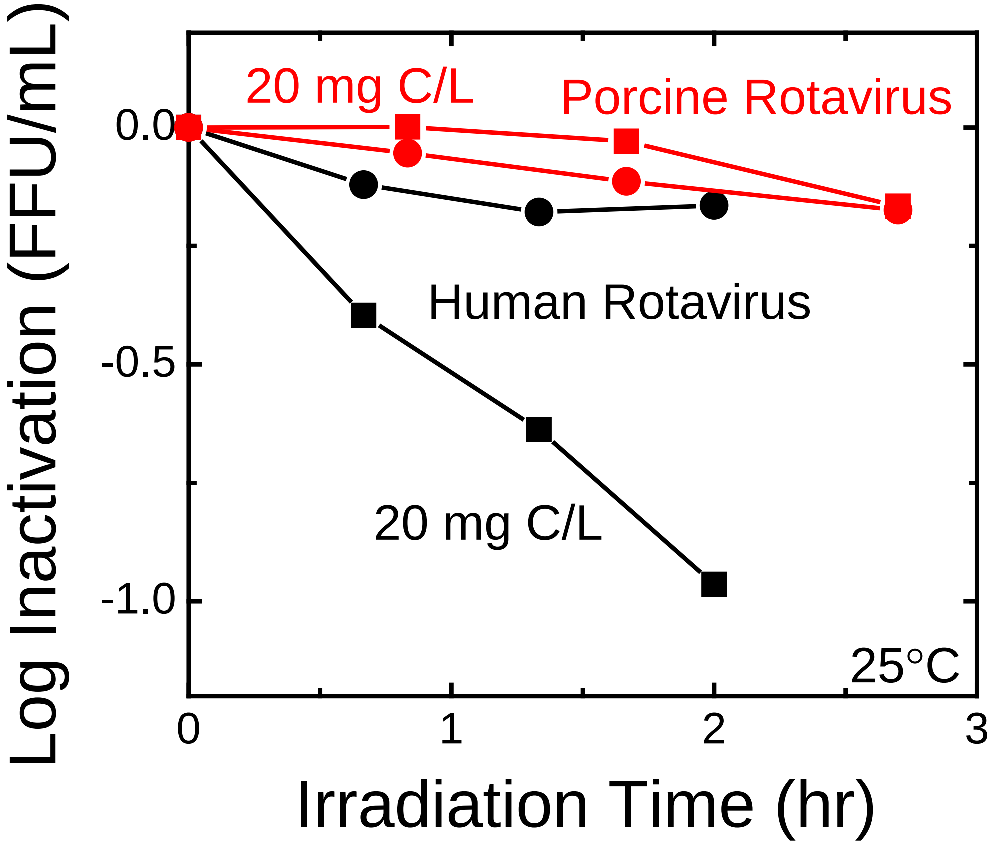
1. The phenotypic and genotypic differences between virus strains can be used to identify the molecular mechanisms responsible for viral susceptibility to solar and UVC inactivation
2. a) Photo-oxidation of viruses depends on the reactions between viruses and reactive radicals formed upon irradiation of wastewater, b) direct effect of UVC irradiation on viruses depends on the irradiation wavelength and the specific UV absorbance (SUVA) of the wastewater
3. Human health risk and life cycle ecological impacts can simultaneously be reduced by leveraging solar disinfection.

Insignificant Endogenous Inactivation of Human and Porcine Rotavirus at 25°C



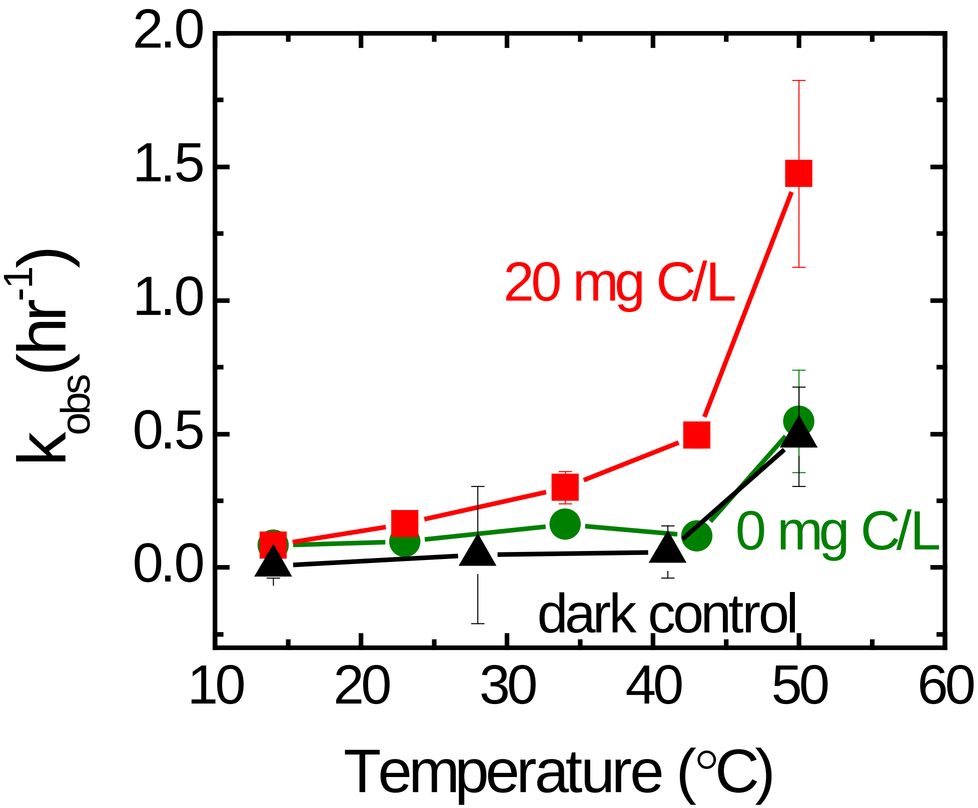
Romero-Maraccini, et al. *ES&T* 2013

Human Rotavirus Is More Susceptible to Exogenous Inactivation by Direct Sunlight UVA than Porcine Rotavirus at 25°C



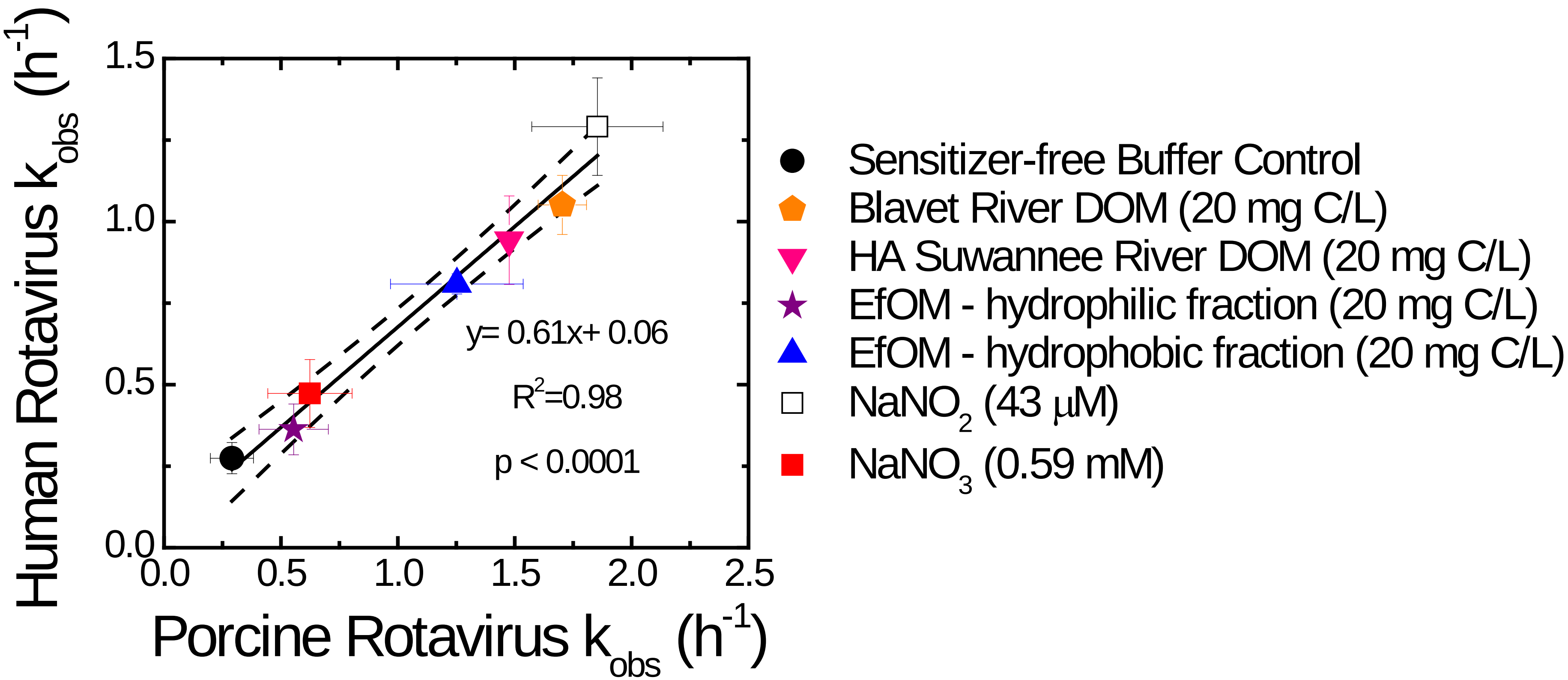
Romero-Maraccini, et al. *ES&T* 2013

Indirect UV-A inactivation of Porcine Rotavirus is important at 40-50ºC



Romero, et al. *ES&T* 2011

Inactivation of Human Rotavirus at 25°C Correlates with Inactivation of Porcine Rotavirus 50°C



Romero-Maraccini, et al. *ES&T* 2013

Reactive Oxygen Species in Natural Water

NO_x^-

$\downarrow$

$^{\bullet}OH$

Hydroxyl radical

$\downarrow$

$^{*}CO_3^{2-}$

DOM $\rightarrow$ $^1DOM^*$

$^3DOM^*$

triplet excited

$\downarrow$

O_2^-

$\downarrow$

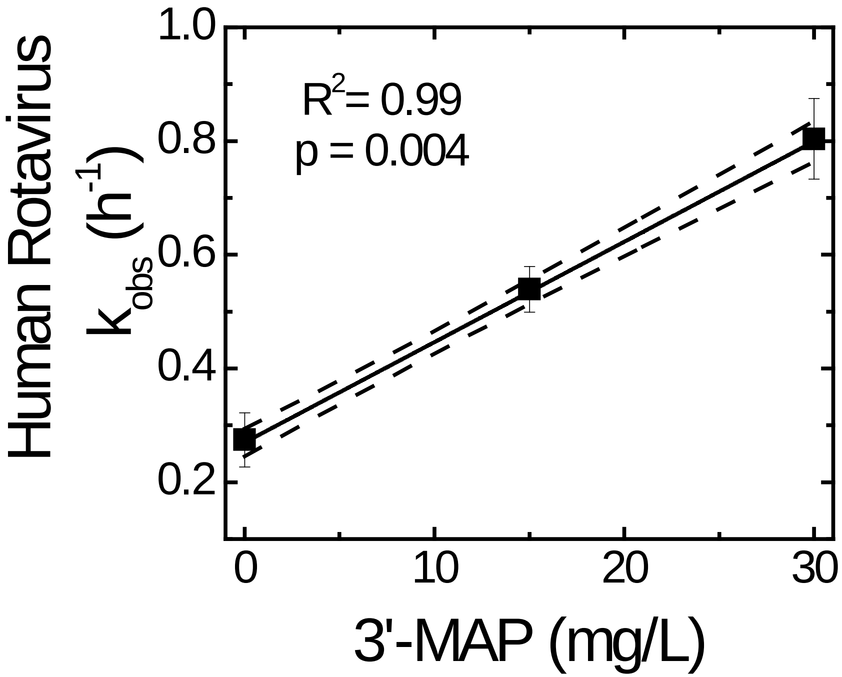
H_2O_2

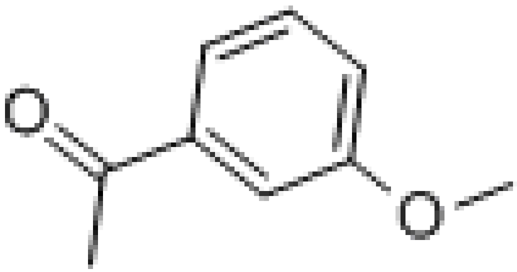
hydrogen peroxide $\rightarrow$ $^{\bullet}OH$ Hydroxyl radical

O_2 $\rightleftarrows$ $^1O_2$

singlet oxygen

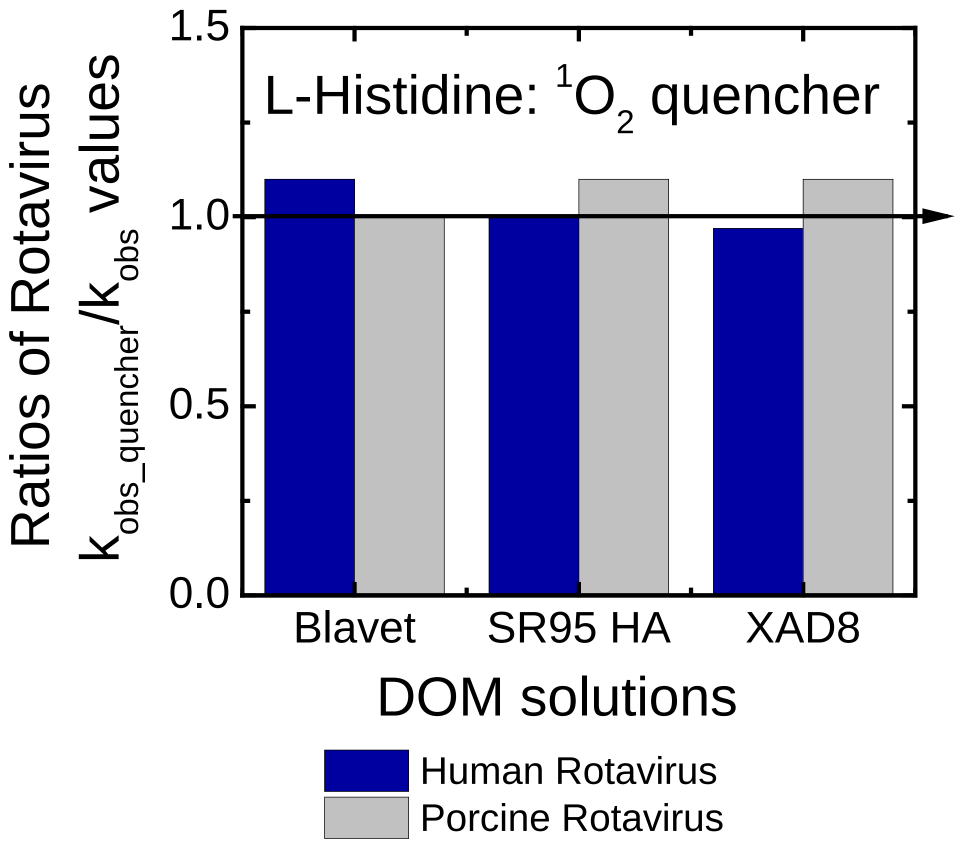
Triplet DOM Can Inactivate Human Rotavirus

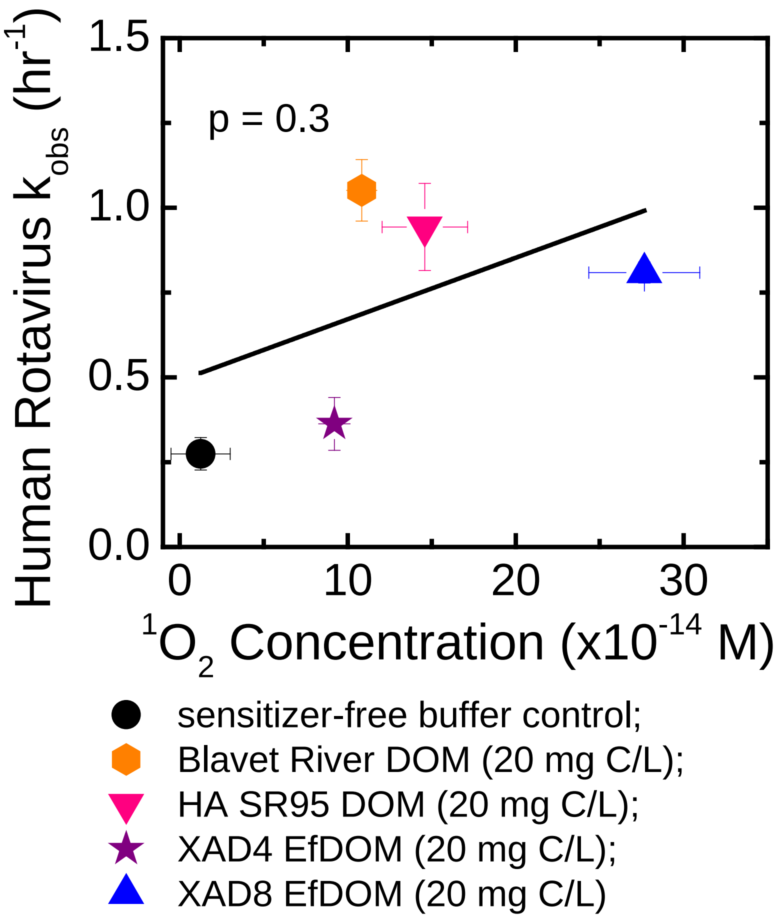




3-Methoxyacetophenone
Triplet DOM* sentisizer

Singlet Oxygen $^1O_2$ Does Not Inactivate Human Rotaviruses





Can H_2O_2 Inactivate Rotavirus?

- 0, 6, and 60 µM of H_2O_2 did not show significant inactivation of rotavirus at 50°C in the dark.
- The measured [H_2O_2] at 50°C in 20 mg/L TOC was ~ 6 µM after the 3 hrs of the experiment.
- Thus, H_2O_2 is not directly responsible for rotavirus inactivation at 50°C.

Need to look at OH radicals

NO_x^-

$\downarrow$

$^\bullet OH$
Hydroxyl radical

$\downarrow$

$*CO_3^{2-}$

$DOM \rightarrow {}^1DOM^*$

$^3DOM^*$
triplet excited

$\downarrow$

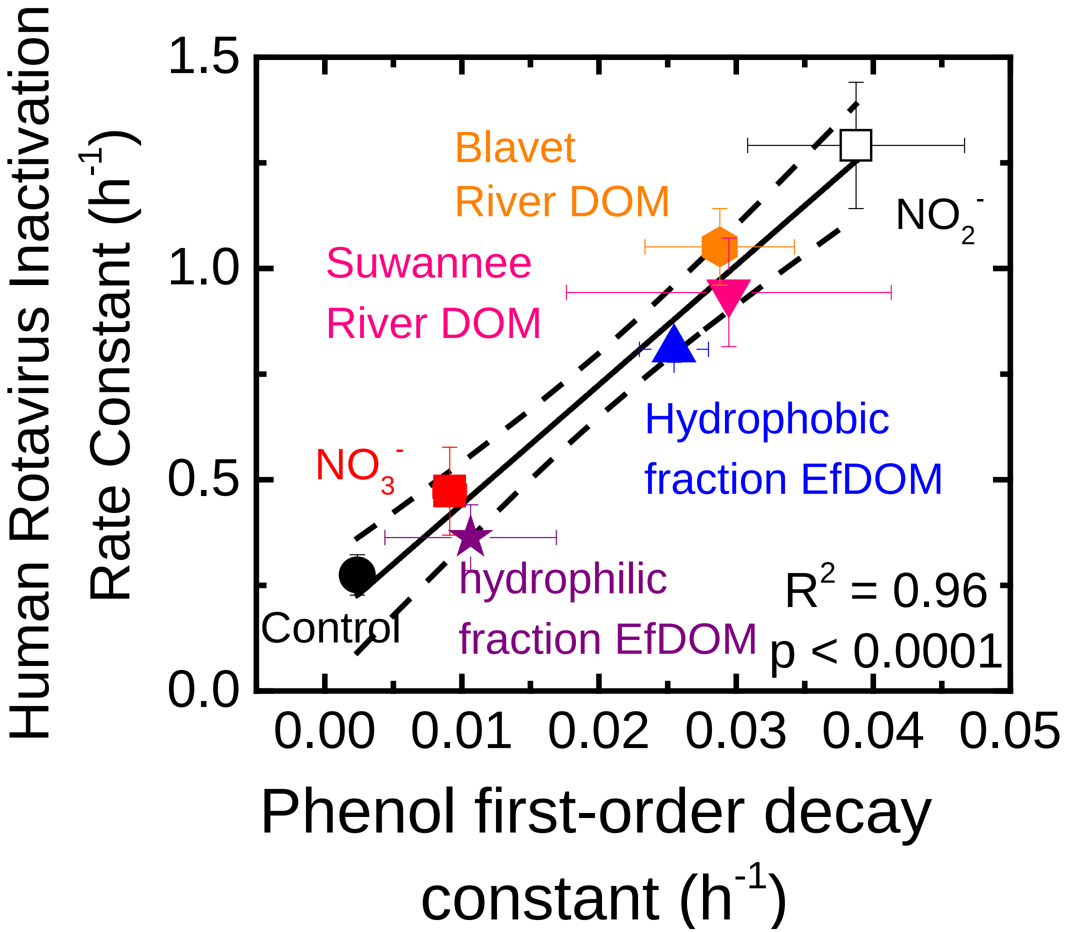
O_2^-

$\downarrow$

H_2O_2
hydrogen peroxide $\rightarrow$ $^\bullet OH$ Hydroxyl radical

$O_2 \rightleftarrows {}^1O_2$
singlet oxygen

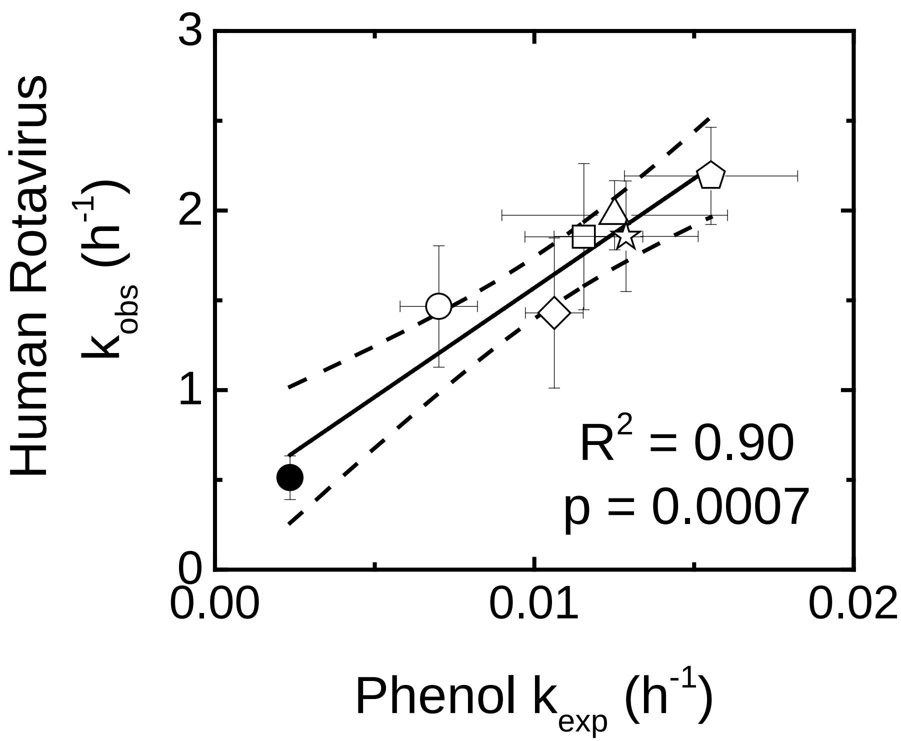
Hydroxyl Radical Is Responsible for Inactivation of Human Rotavirus Wa: Hypothesis 2a Was Proven



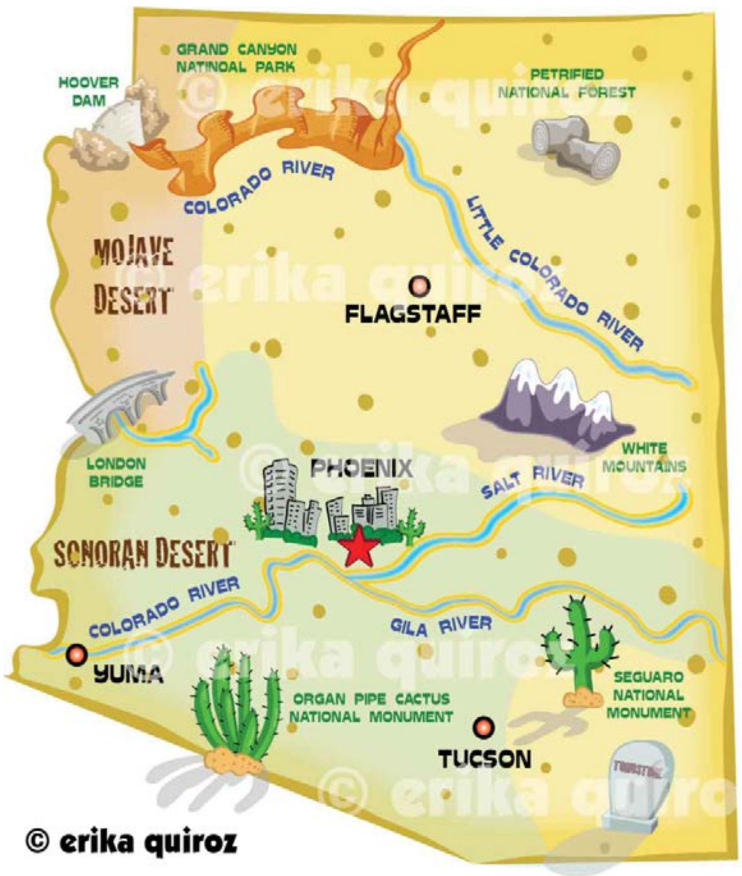
pH=8.0 and 25ºC

Romero-Maraccini, et al. *ES&T* 2013

Indirect UVA Inactivation of Human Rotavirus In Waste Stabilization Pond Water



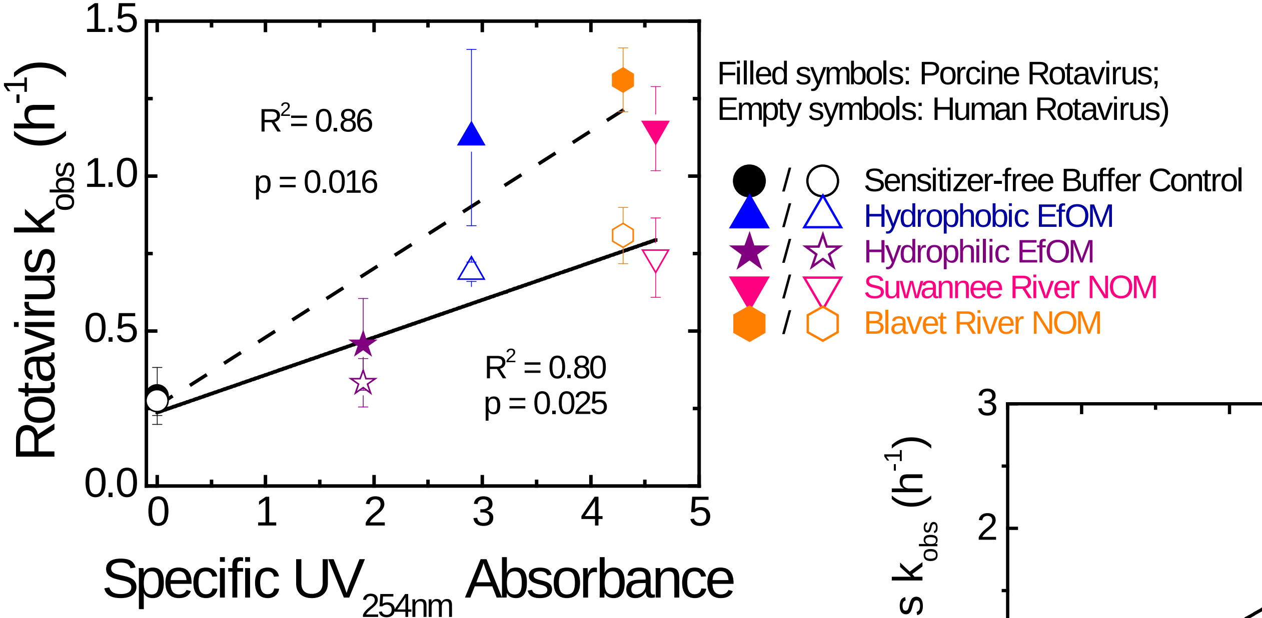
● Buffer Control; ○ Pond 1; □ Pond 2
△ Pond 3; ◇ Poind 4; ⬠ Pond 5; ☆ Pond 6

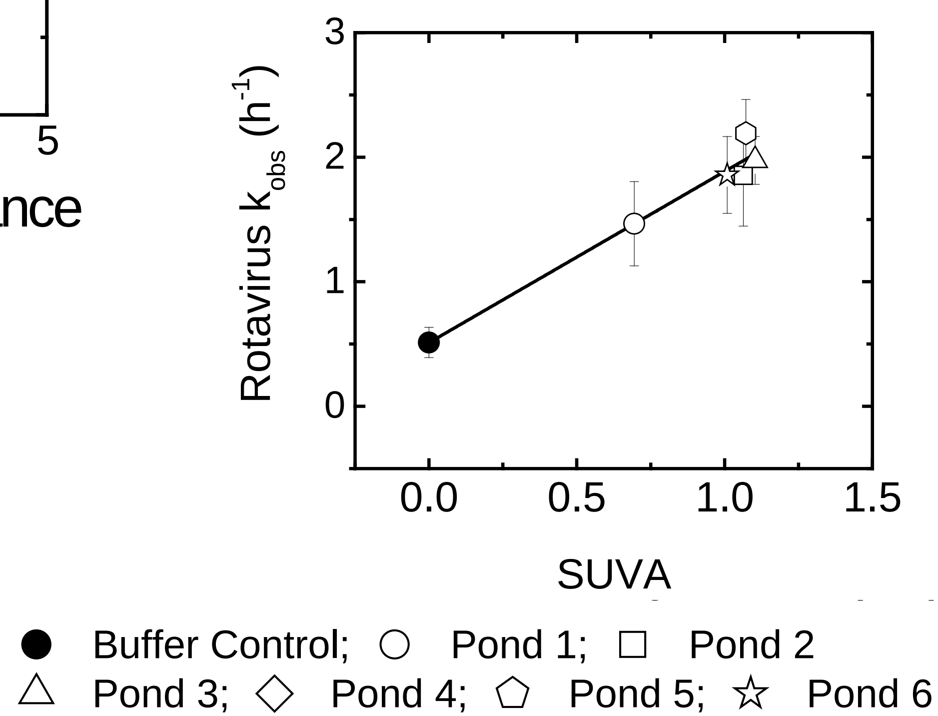


http://erikaquiroz.com/map-of-arizona/

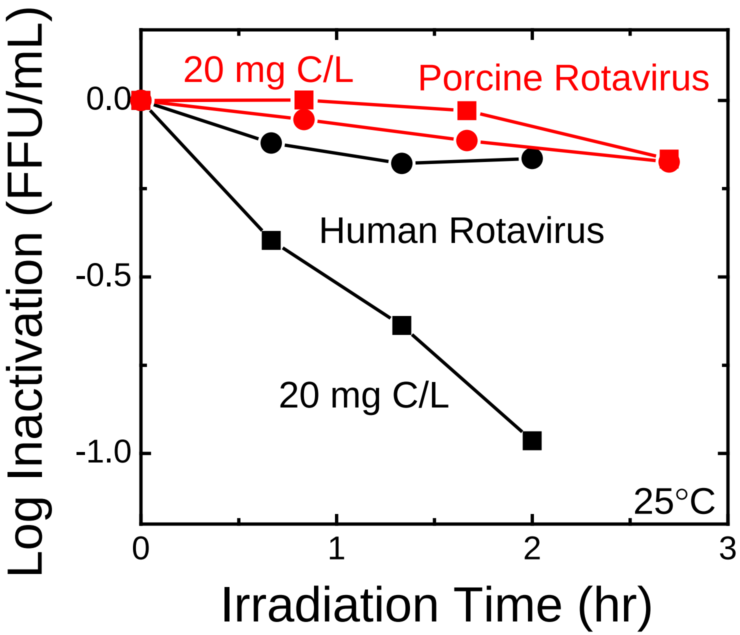
pH=8.0 and 25ºC

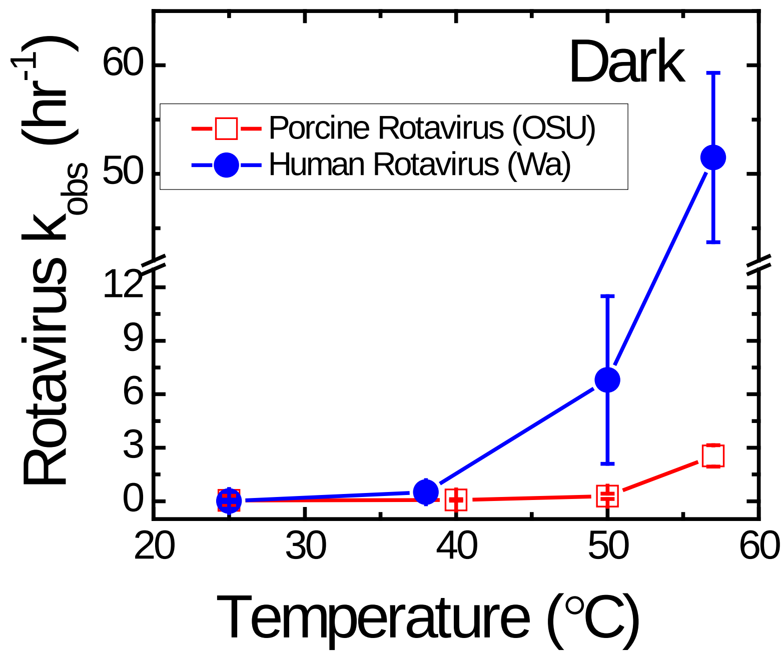
Inactivation Rate Constants Correlated with SUVA





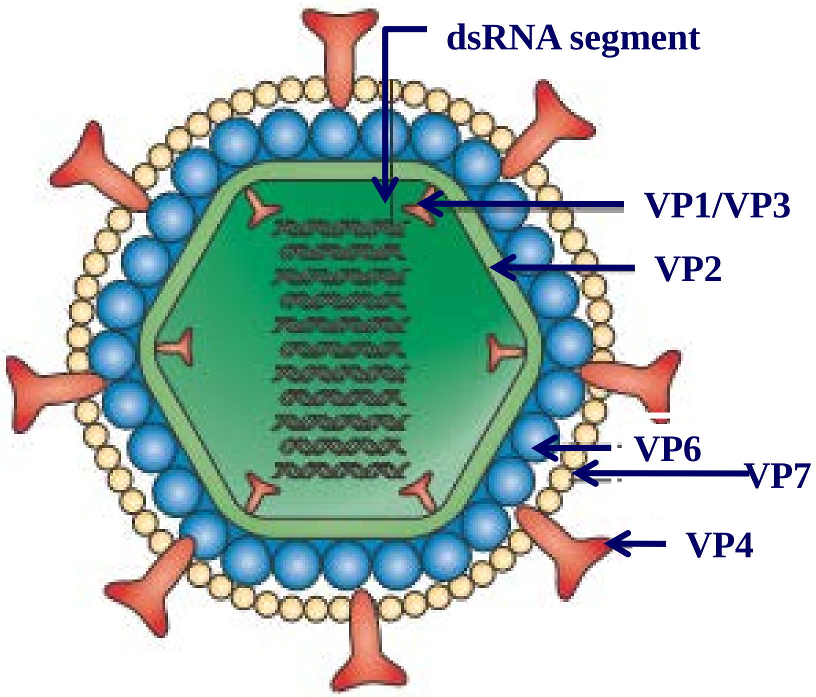
Porcine Rotavirus Is More Resistant Than Human Rotavirus





Romero-Maraccini, et al. *ES&T* 2013

Significant Differences in Structural Protein Sequences of Human RV Wa and Porcine RV OSU



Manuel A. *et al*., 2007.

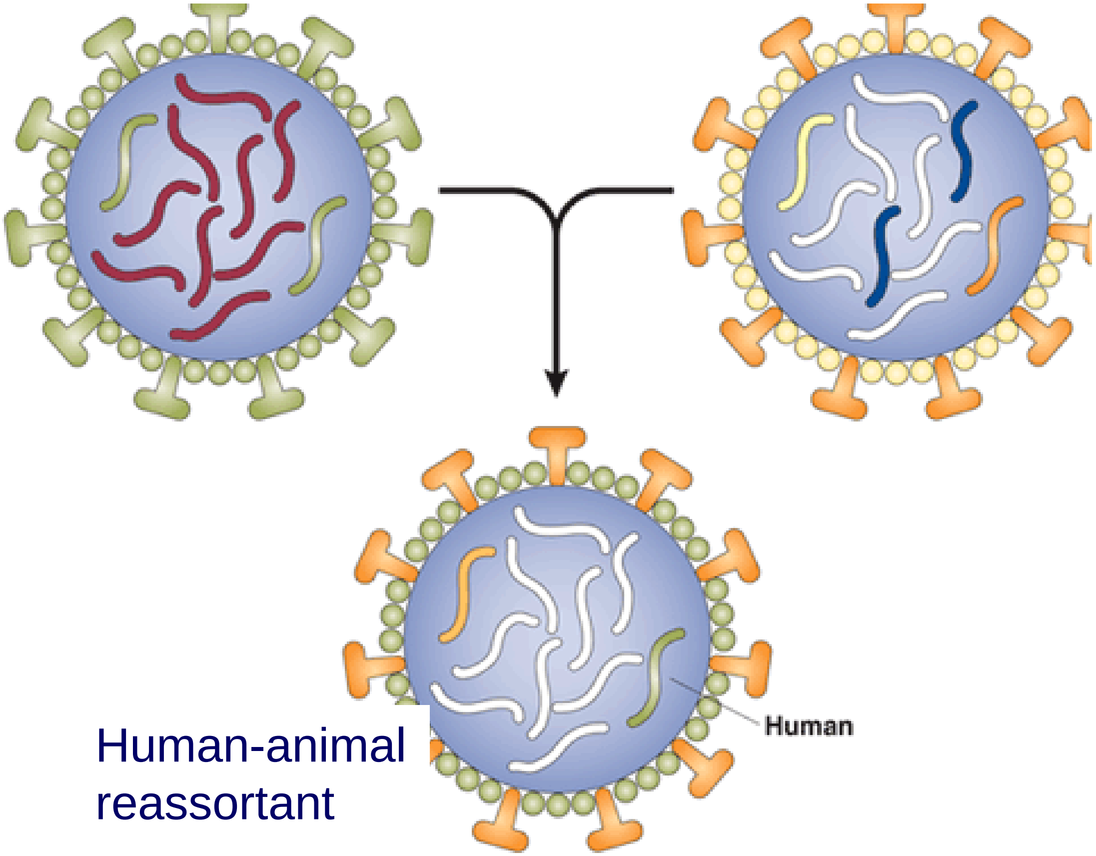
Protein	% identity of OSU to Wa
VP1	97
VP2	97
VP3	94
VP4 *	69
VP5	75
VP8	54
VP6	93
VP7	79

Romero-Maraccini, et al. *ES&T* 2013

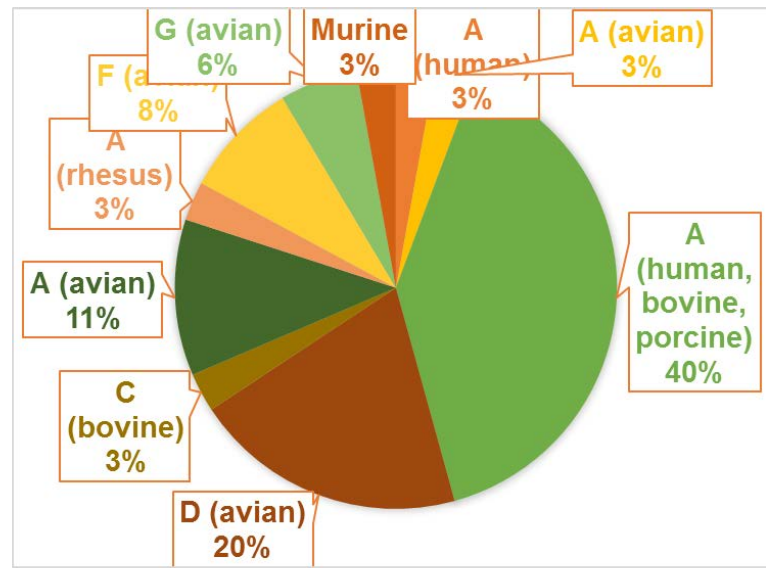
Rotavirus Reassortment

Human Rotavirus

Animal Rotavirus



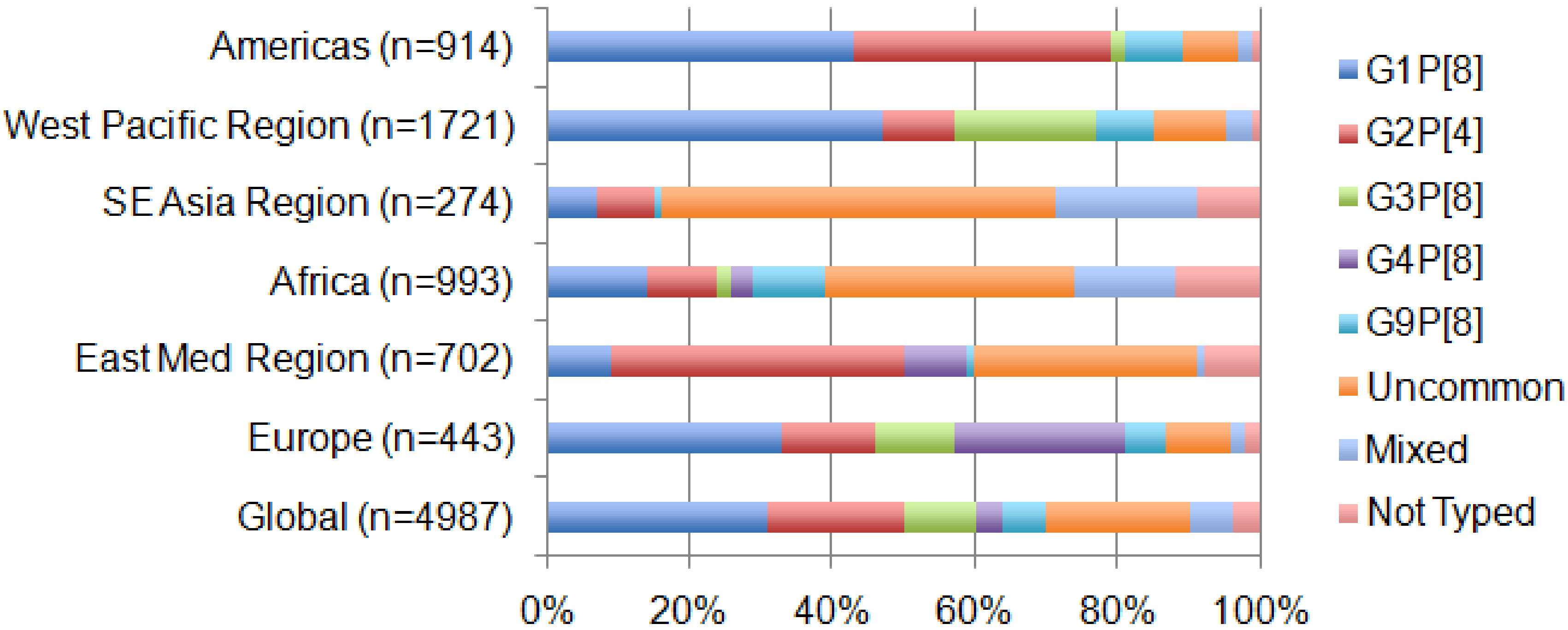
Human-animal reassortant



Aw, et al., International Journal of Food Microbiology, 223, 16 April 2016

Modified after Barry C Buckland, *Nature Medicine* **11**, S16 - S19 (2005)

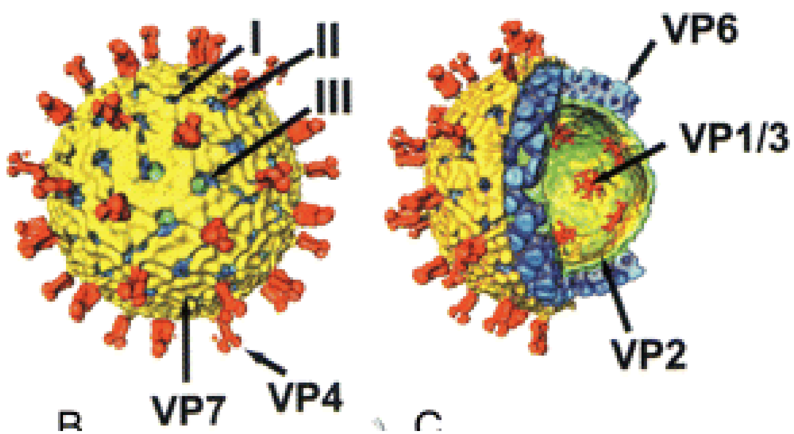
Distribution of RV Genotypes Reported to the WHO Surveillance Network in 2010



John-T-Patton, Rotavirus diversity and evolution in the post vaccine world, Discovery Medicine, 2012

Studied Rotavirus Serotypes

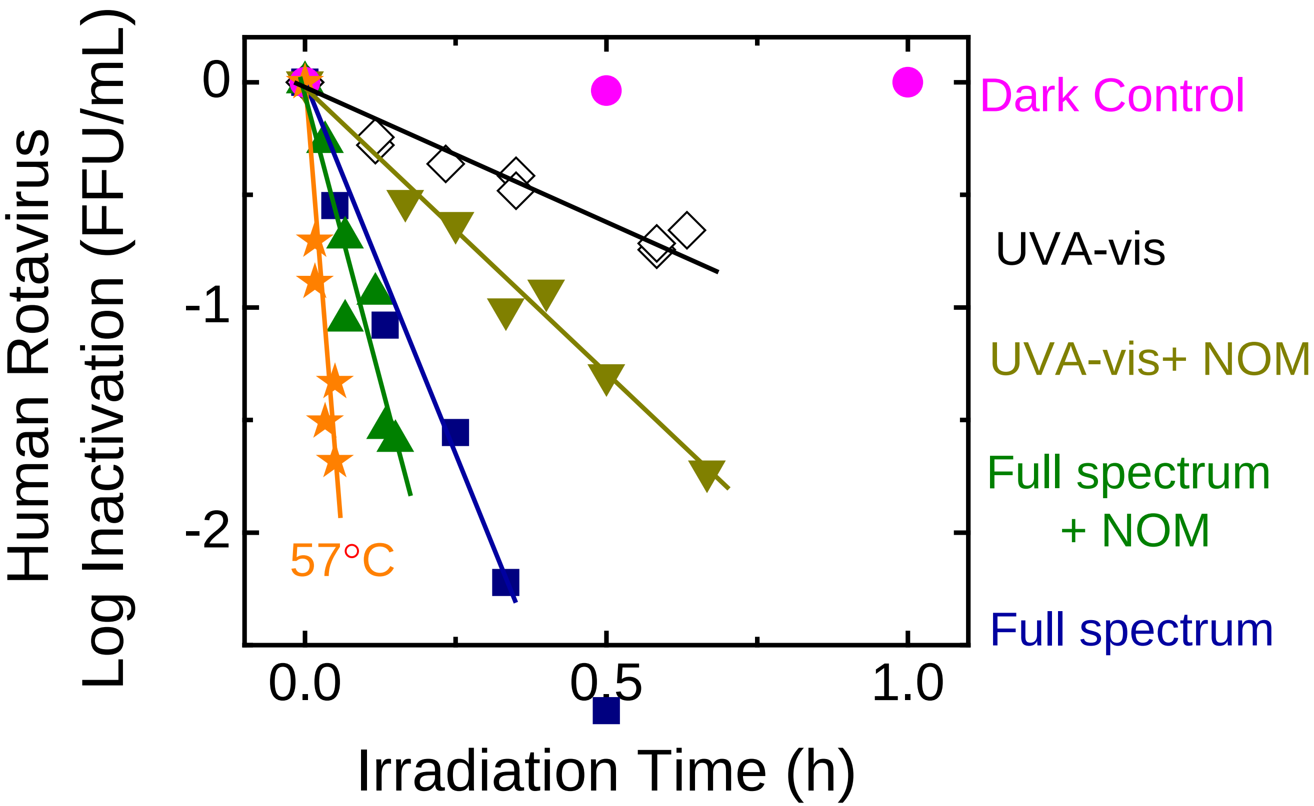
RV Glycoprotein	Strains	Host
G1	Wa	Human
G2	S2	Human
G3	YO	Human
G4	ST3	Human
G5	OSU	Porcine

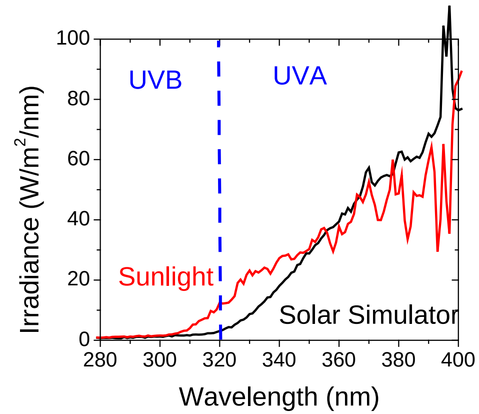


- VP6 : 7 serological groups (A to G)
- VP7 (glycoprotein) :14 G serotypes

Desselberger, et al.(2009) Rotavirus and rotavirus vaccines. British Med Bull (2009) 90 (1)

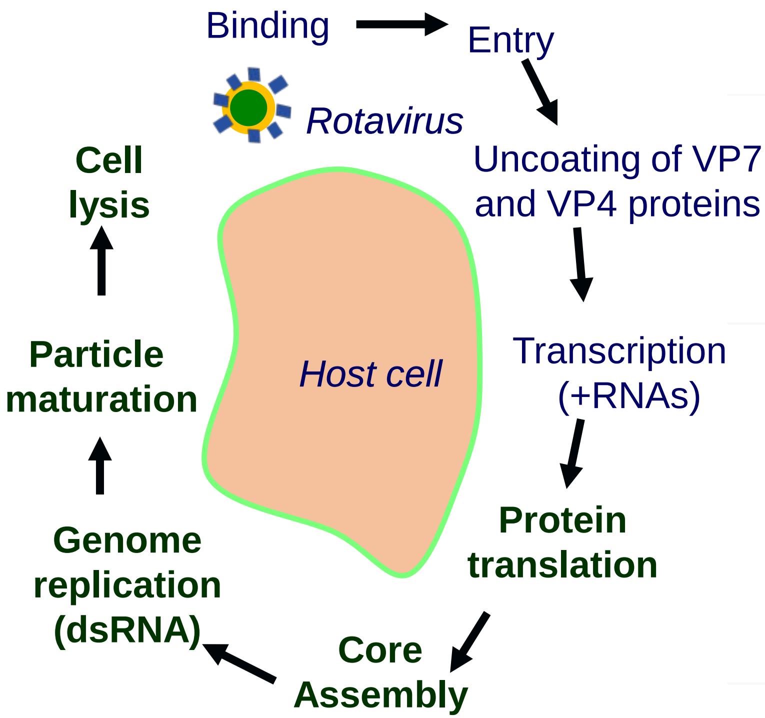
Effects of Solar Irradiation and Temperature Treatments on the Life Cycle of Human Rotavirus Wa





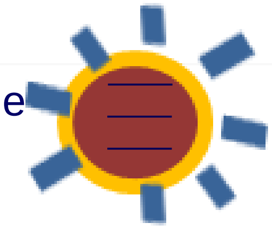
Romero-Maraccini, et al. *AEM,* 2015

Rotavirus Life Cycle



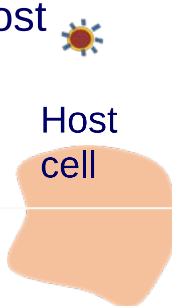
Genome integrity

- Targeted a portion of the NSP3 segment



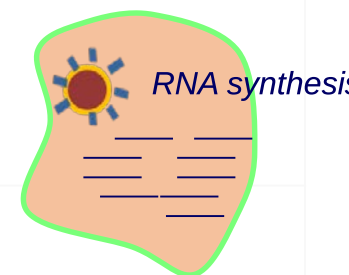
Binding ability

- Tracked bound rotavirus to host cell receptors by quantifying the NSP3 gene by RT-PCR



RNA synthesis

- Assessed RNA synthesis by quantifying the levels of NSP3 gene copies generated inside the host by RT-PCR



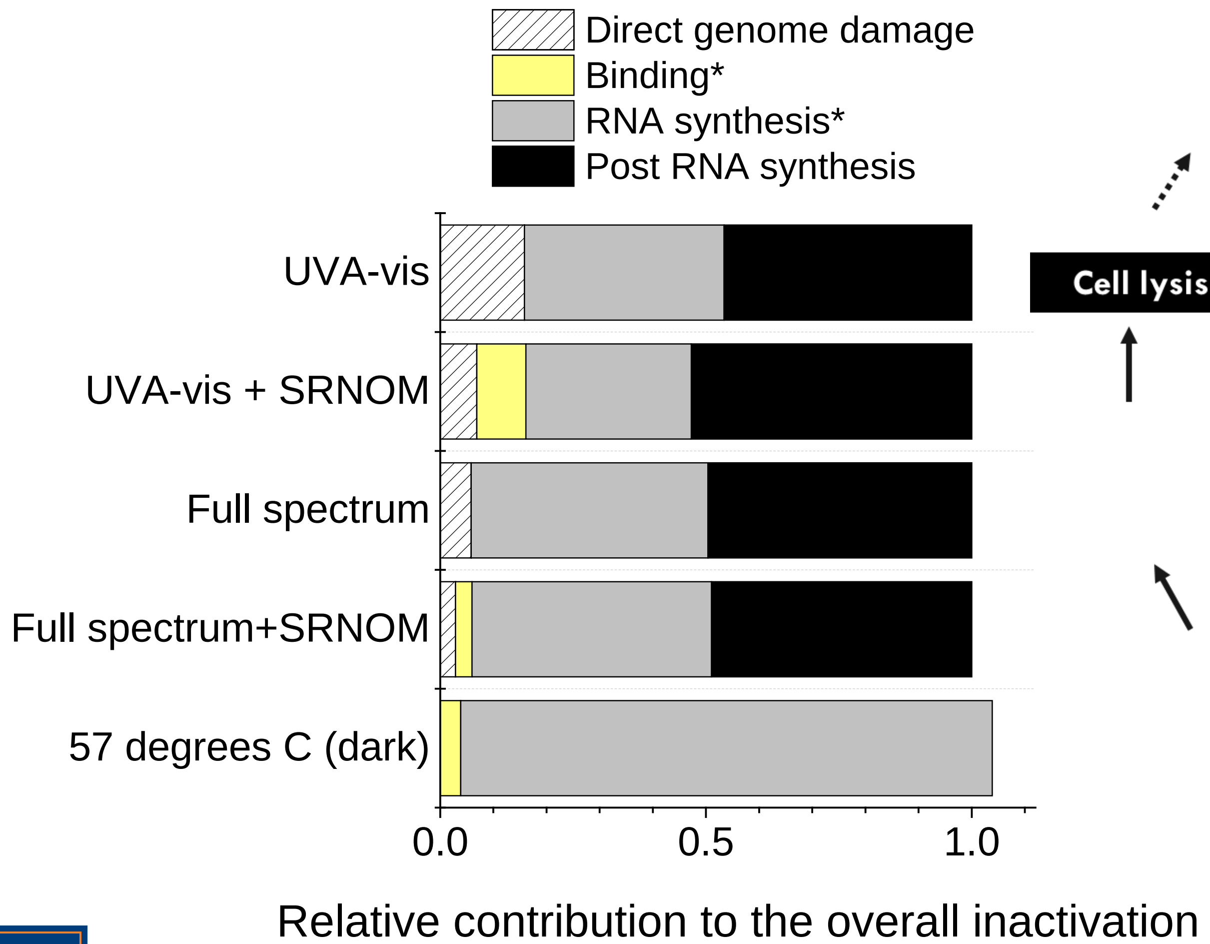
Cell lysis

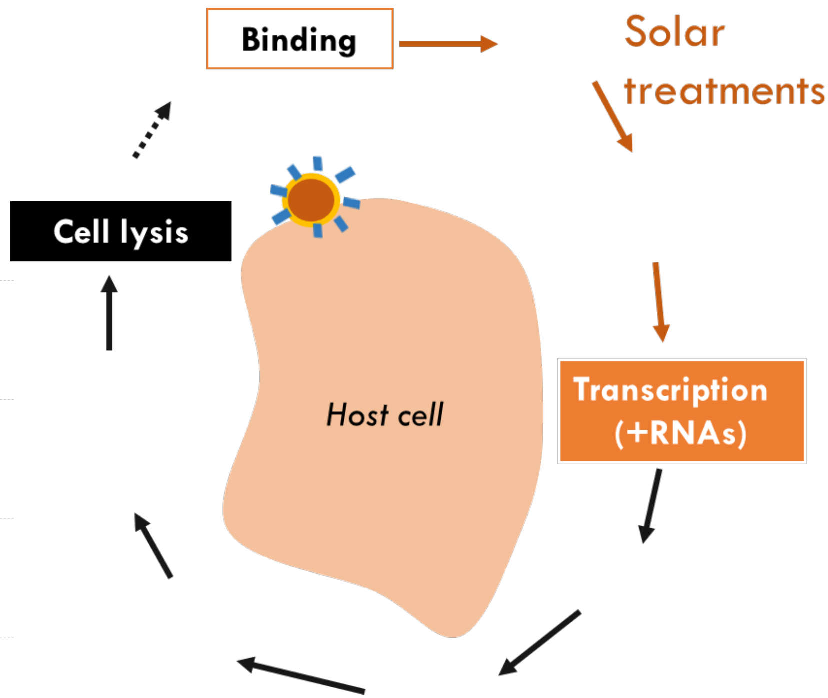
- Conducted the infectivity assay


Infected MA-104 cells

Romero-Maraccini, et al. *AEM,* 2015

Effects of Treatments on RV Life Cycle





Romero-Maraccini, et al. *AEM* 2015

Year 1 Summary

1. Hydroxyl radical produced by organic matter triggers rotavirus Wa inactivation (hypothesis 2a is proven for one strain).
2. Genetically different strains of rotavirus have different susceptibility toward solar disinfection.
3. When using solar disinfection, the decrease in RNA synthesis was responsible for approximately one-half of the decrease in infectivity, suggesting that other mechanisms, including posttranslational, contribute inactivation.

Year 2 and 3 Plan

1. Determine the molecular mechanisms responsible for inactivation of different RV strains;
2. Determine factors required for effective virus inactivation by UVC; and
3. Develop pond and UVC design guidelines to achieve reliable virus inactivation and elucidate trade-offs across and within dimensions of sustainability.

Publication Supported by EPA Project RD83582201-0

- Romero-Maraccini, OC, Shisler, JL, Nguyen., TH., Solar and Temperature Treatments Affect the Ability of Human Rotavirus Wa To Bind to Host Cells and Synthesize Viral RNA, Applied and environmental microbiology, 81 (12), 4090-4097, 2015, doi:10.1128/AEM.00027-15.
- Feng, Z., Lu, R., Yuan, B., Zhou, Z., Wu, Q., Nguyen, T.H., Influence of solution chemistry on the inactivation of particle-associated viruses by UV irradiation, Colloids and Surfaces B: Biointerfaces, 148, pp. 622–628, 2016, doi: j.colsurfb.2016.09.025.
- Fuzawa, M., Ku, K.-M., Palma-Salgado, S. P., Nagasaka, K., Feng, H., Juvik, J. A., Sano, D., Shisler, J.L., and Nguyen, T. H., Effect of Leaf Surface Chemical Properties on the Efficacy of Sanitizer for Rotavirus Inactivation, Applied and Environmental Microbiology, 82 (20), pp 6214-6222, 2016, doi: 10.1128/AEM.01778-16