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Lewis Katz School of Medicine

Elimination of Human Pathogenic Viruses by Anthem One UV-C Irradiation

<u>INTRODUCTION</u>: Anthem One UV-C light has been examined for its capability for eradication of human pathogenic viral agents including members of Coronaviruses, Flaviviruses, and Retroviruses. We demonstrated that 10-20 seconds exposure of human coronavirus OC-43, Zika Virus (ZIKV), and HTLV-1 viral stocks to Anthem One UV-C light completely eliminates viral infectivity in cells in culture. Test results were provided by both Temple and George Mason Universities.

<u>Viral strains and Institutions</u>: Experiments related to Zika Virus (PRVABC59) were performed and analyzed at the Department of Neuroscience in Lewis Katz School of Medicine, Temple University, Philadelphia by Drs. Kamel Khalili and Ilker K. Sariyer. Experiments related to the Beta-coronavirus OC43 and HTLV-1 were performed and analyzed at George Mason University College of Science by Dr. Fatah Kashanchi. All procedures and experiments were reviewed and approved by the Institutional Biosafety Committees (IBC).

<u>UV-C light source and instrument</u>: We used and tested Anthem One Mark Two UV-C 265nm Medical Grade Sterilizer LED Light as the UV light source for the pre-treatment of viral stocks.

Rationale: UV light has been proposed and used for its capability of reducing pathogenicity of a variety of pathogens including viruses. We investigated this further by pre-exposing ZIKV viral stocks to UV light before infecting primary human astrocytes. Primary goal of the experiment was to determine if the proposed instrument as the UV source can be utilized for reducing pathogenicity of RNA viruses with close structural features to 2019-nCoV and adapted to the clinical settings for prophylaxis purposes.

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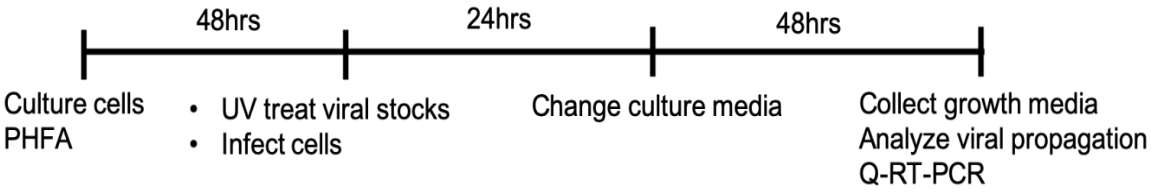
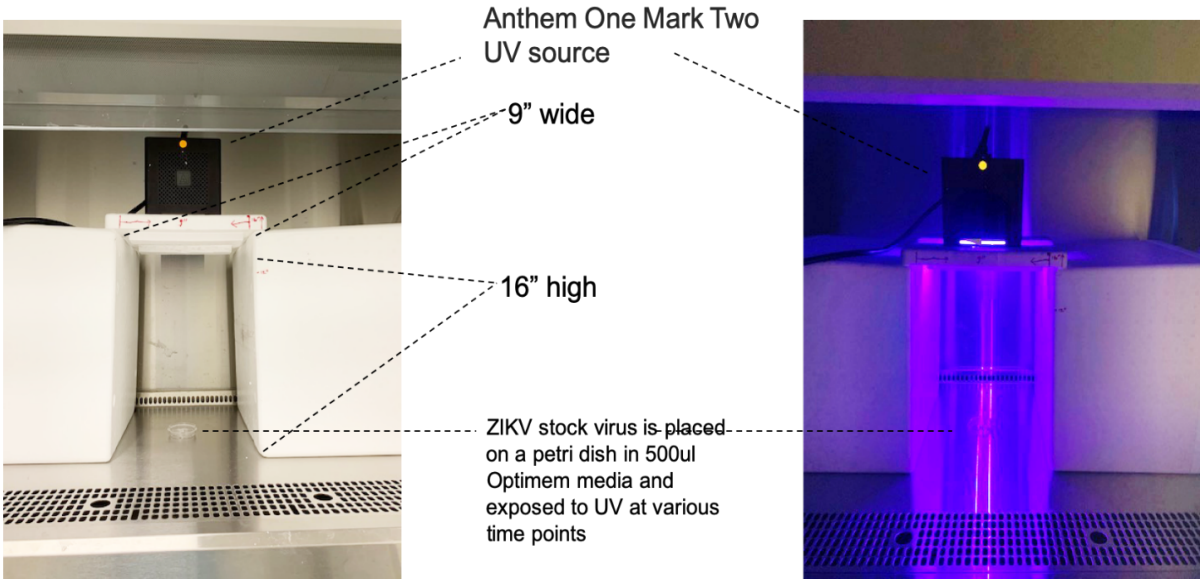
UV light source: Anthem One Mark Two UV-C 265nm Medical Grade Sterilizer LED Light is a UV light source that we used in our BSL2+ facilities.

EXPERIMENTAL SETUP





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Experimental setup and methodology: Five hundred microliters of ZIKV (PRVABC59) viral stocks in opti-MEM media were exposed to UV using the 100% mark at 16 inches for various time points (0, 5sec, 10sec, 15sec, 30sec, 45sec, 1min, 5min, 10min, 20min, and 30min) in the BSL2+ settings in the biosafety cabinet by following BSL2+ guidelines. Primary human fetal astrocytes (PHFA) were cultured to confluency of 70% in 12-well tissue culture dishes. Each well had $0.5x10^6$ cells. UV exposed ZIKV stocks were then used to infect PHFA cells. Infection was performed at 1MOI. Culture media were changed at 24hrs post infections. Growth media of cells were collected at 3dpi and processed by qRT-PCR for the detection and quantification of released viral particles in culture media. Experiments were performed in duplicate and analyzed with duplicate internal repeats. One hundred microliters of OC-43 viral stocks in either DMEM media or saliva were irradiated using the "50% mark" at 12 inches for 20 seconds in the BSL2+ settings in the biosafety cabinet by following BSL2+ guidelines. The UV treated viral samples were then added to VeroE6 or Lung primary cells for 72 hours followed by qRT-PCR for any potential growth of the virus. One hundred microliters of HTLV-1 infected supernatants from MT-2 cells (~ 10^7 viral RNA copy number) were added to either RPMI media or saliva (1:2 dilution) and then treated with UV prior to culture with CEM (a T-lymphoblastic leukemia cell line), recipient cells. Co-cultures were carried for 72 hours and total RNA was isolated for qRT-PCR for any potential growth of the virus.

Results:

PHFA cells infected with untreated ZIKV stocks showed a robust cytotoxicity as evident by excessive floating death cells with bright and round appearance. UV exposure at five and ten second time points had a reduced but visible ZIKV cytotoxicity. The morphology of PHFA cells at fifteen second and further exposure time points were undistinguishable from uninfected controls and showed no evidence of cytotoxicity. Consistent with the morphological assessment of the cells, pre-UV exposure of ZIKV stocks showed a dramatic reduction in viral propagation rates assessed by q-RT-PCR analysis starting at 15 sec and further exposure time points. Beta-coronavirus OC43 showed a productive infection in recipient Vero and Lung primary cells and pre-UV-C exposure of the virus for 20 seconds both in DMEM media and saliva resulted in a dramatic reduction in viral propagation. Similarly, while control/untreated HTLV-1 stocks showed a productive infection in CEM cells, pre-UV-C exposure of the virus in either RPMI media or saliva caused almost complete loss in viral infectivity.

Conclusions: Our results suggest that UV-C exposure effectively inactivates ZIKV, Beta-coronavirus OC-43, and HTLV-1 infectivity and perhaps several other RNA viruses.

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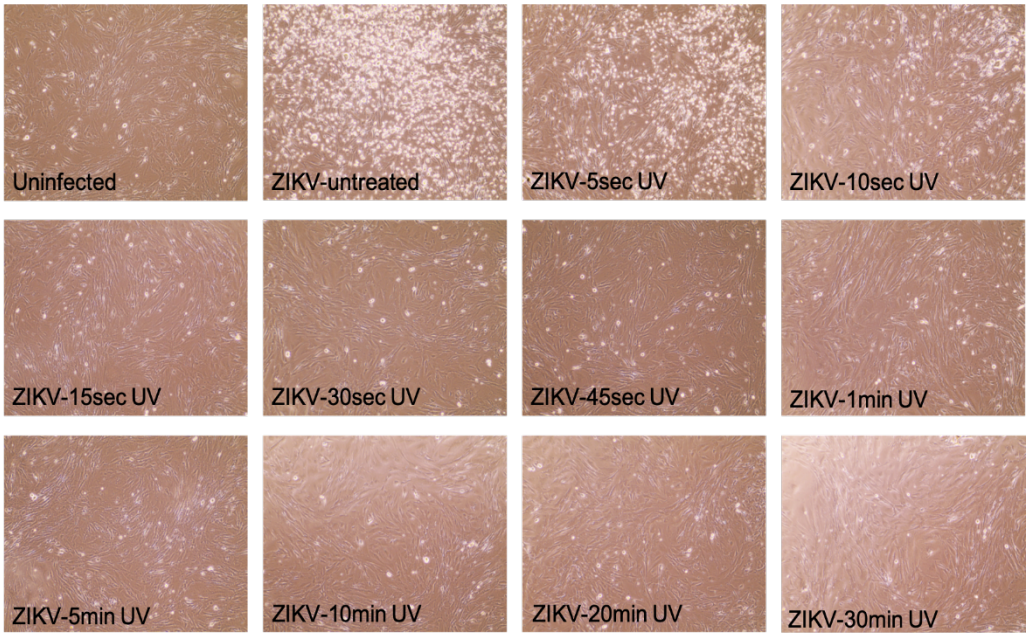
Figure 1: Morphological assessment of pre-UV treatment of ZIKV on cytotoxicity in PHFA cells: Phase contrast images from uninfected and ZIKV infected PHFA cells (either untreated or exposed to UV at various time points) were taken at 3dpi. PHFA cells infected with untreated ZIKV stocks showed a robust cytotoxicity as evident by excessive floating dead cells with bright and round



appearance. At five and ten second UV exposure time points, cells exhibited a reduced but visible ZIKV cytotoxicity. The morphology of PHFA cells at fifteen second and further exposure time points was undistinguishable from uninfected controls and showed no evidence of cytotoxicity. These results suggest that fifteen second UV exposure is sufficient to inactivate ZIKV viral particles and prevent the cytotoxicity associated with viral replication.

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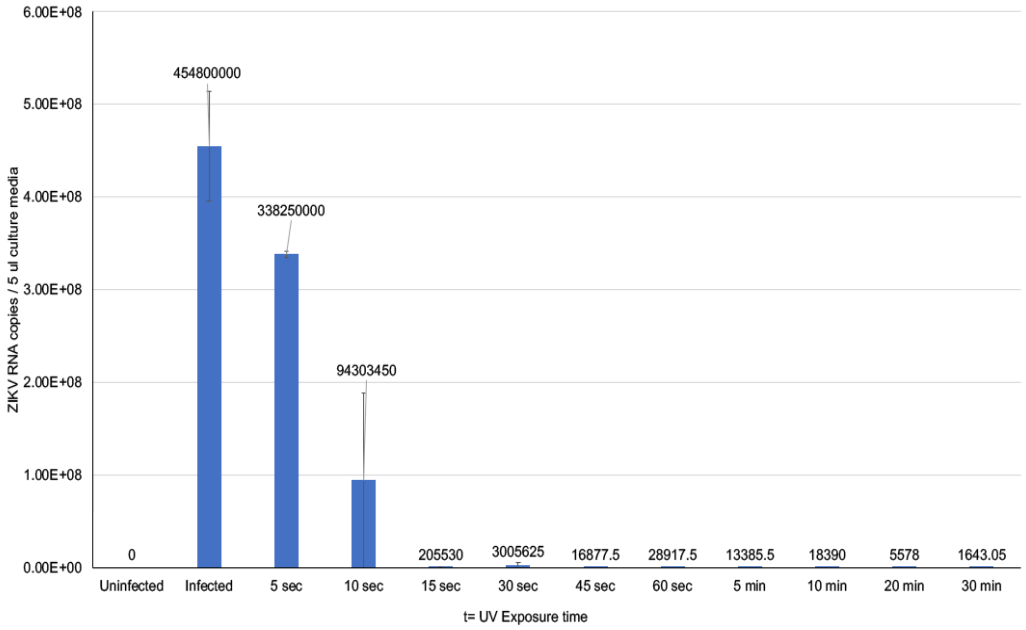
Figure 2:



Quantitative analysis of ZIKV replication after UV-C exposure in PHFA cells. PHFA cells were infected with ZIKV either untreated or pre-exposed to UV light at various time points. At 3 dpi, growth media of the cells were collected and analyzed by real time q-RT-PCR and shown as bar graphs. Consistent with the morphological assessment of the cells (Fig.1), pre-UV exposure of ZIKV stocks ***showed a dramatic reduction in viral propagation rates starting at 15 sec and further exposure times.*** (Note: In the q-RT-PCR, baseline RNA copies are expected to be detected due to the infection of cells with stock virus at 1MOI.)

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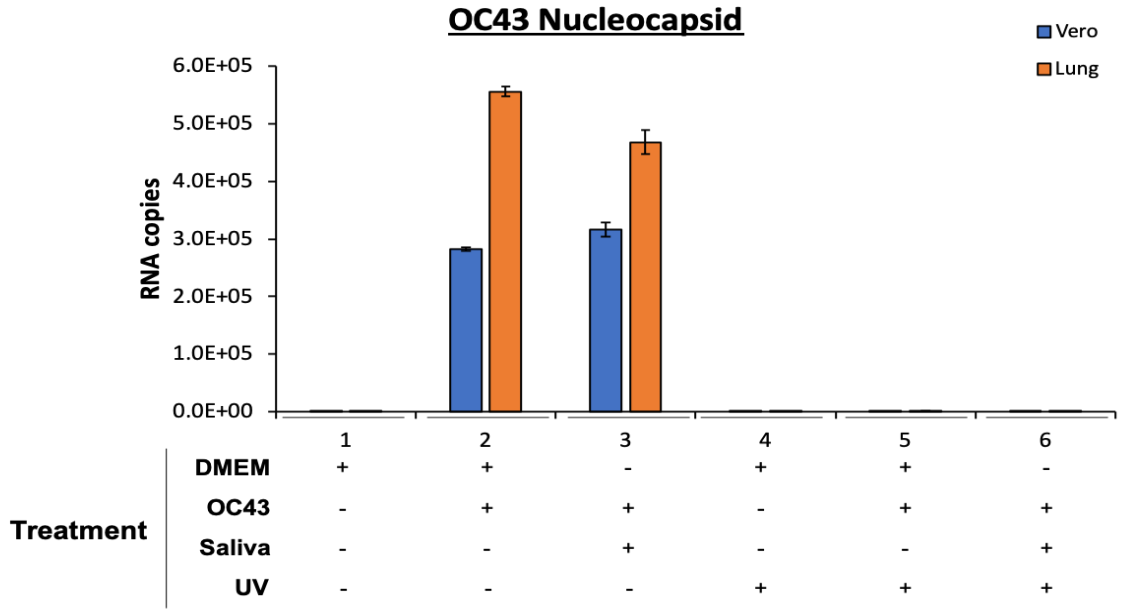
Figure 3: Quantitative analysis of Beta coronavirus OC43 infectivity after UV-C



exposure in Vero cells and primary lung cells. One hundred microliters of OC43 viral stocks in either DMEM media or saliva were irradiated using the "50% mark" at 12 inches for 20 seconds in the BSL2+ settings in the biosafety cabinet by following BSL2+ guidelines. The UV treated viral samples were then added to VeroE6 or Lung primary cells for 72 hours followed by qRT-PCR for any potential growth of the virus. Beta-coronavirus OC43 showed a productive infection in recipient Vero and Lung primary cells and ***pre-UV-C exposure of the virus for 20 seconds both in DMEM media and saliva resulted in a dramatic reduction in viral propagation.***

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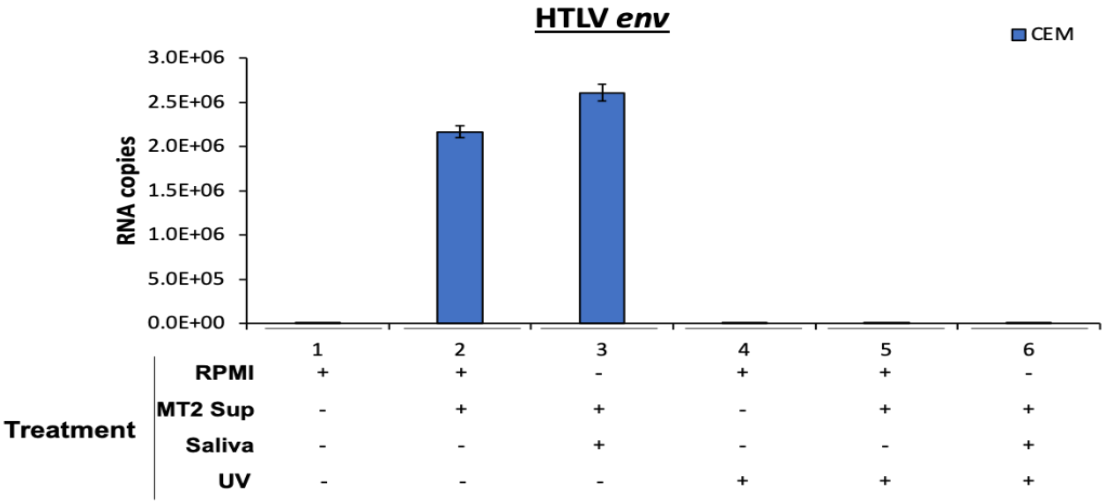
Figure 4: Quantitative analysis of HTLV-1 replication after UV-C exposure in CEM cells. One hundred microliters of HTLV-1 infected supernatants from MT-2 cells (~ 10^7 viral RNA copy number) were added to either RPMI media or saliva (1:2 dilution)



and then treated with UV prior to culture with CEM recipient cells. Co-cultures were carried for 72 hours and total RNA was isolated for qRT-PCR for any potential growth of the virus. Interestingly, while control/untreated HTLV-1 stocks showed a productive infection in CEM cells, ***pre-UV-C exposure of the virus in either RPMI media or saliva caused almost complete loss in viral infectivity.***

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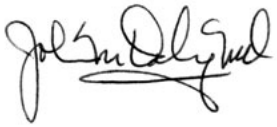
<u>Summary Conclusions:</u> Anthem One Mark II effectively inactivates ZIKV, Beta-coronavirus OC-43, and HTLV-1 infectivity and perhaps several other RNA viruses. We demonstrated that 10-20 seconds exposure of human coronavirus OC-43, Zika Virus (ZIKV), and HTLV-1 viral stocks to Anthem One UV-C light completely eliminates viral infectivity in cells in culture.

For further discussion of the results, please contact Drs Khalili and Kashanchi directly.

Sincerely,



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