College of Medicine
Department of Medicine

Institute for Therapeutic Innovation
Research and Academic Center
6550 Sanger Road
Orlando, FL 32827
407-313-7068
407-313-7095 Fax

University of Florida Evaluation of the Puradigm FLOW against SARS-CoV-2, the causative agent of COVID-19

Study was performed June 24 – June 25, 2020
Viral Titration Assay was conducted June 26, 2020
Results were read June 29, 2020

Purpose: To determine if the Puradigm FLOW technology inactivates a human infectious SARS-CoV-2 isolate, the virus that is responsible for causing COVID-19.

Experimental Design:
The human SARS-CoV-2 isolate USA-WA1/2020 was inoculated onto 20 stainless steel coupons as 10^5 plaque forming units (PFU) in 0.1 ml. The viral inoculum was allowed to dry onto each coupon. Two coupons were harvested immediately and processed, serving as an initial inoculation control (0 h time point). Half of the remaining 18 coupons served as controls and were incubated at room temperature in the absence of the FLOW technology. The other 9 coupons were incubated in a GERMFREE custom class III biosafety cabinet that housed the FLOW unit. Coupons inoculated with 10^5 PFU of SARS-CoV-2 were harvested from the control and FLOW treated groups at 4 h, 6 h, and 24 h post-exposure to the FLOW technology in triplicate (3 controls and 3 FLOW treated coupons at each time point for a total of 6 samples per time point). Following collection, coupons were placed in 50 ml conical tubes containing 5 mls of Minimal Essential Medium (MEM). Following vigorous vortexing, coupons and medium were frozen at -80°C until the end of the study. Infectious SARS-CoV-2 was quantified by plaque assay on Vero E6 cells for all samples simultaneously to avoid assay to assay variability. The assay limit of detection was 1.4 $\log_{10}$ PFU.

An ozone meter was placed inside the Class III biosafety cabinet to ensure ozone levels remained below 0.05 ppm throughout the duration of the experiment. In order to replicate real world conditions, the exhaust port was opened to allow for ventilation approximately once every 2 hours, equating to less than 1 air change per hour (by comparison a typical patient room will have at least 4 air changes per hour [ASHRAE Standard 62-2001, Appendix E]). Ozone levels never exceeded 0.04 ppm during the experimental procedure.

The experimental results are shown in Figure 1. SARS-CoV-2 decreased in infectivity naturally as the infectious viral burden steadily declined in the control group regardless of the starting viral inoculum. Measurable viral titers were achieved in the control arm throughout the entire experiment.

After 4 h of incubation on the coupon, SARS-CoV-2 decreased in viral titer by 7.9-fold in the control arm, whereas the FLOW treated coupons resulted in a 398.1-fold reduction in infectious viral burden at the same time point. By 6 h post-viral inoculation onto the coupon, viral burden further decreased by an additional 1.6-fold in both experimental groups, resulting in an overall reduction of 12.6-fold in the control and 631.0-fold in the FLOW treated samples, relative to the time 0 measurement. By 24 h, the FLOW exposed coupons did not have any detectable levels of infectious SARS-CoV-2; however, 2.1-$\log_{10}$ PFU of virus were detected in the matched control arm.

Taking into account the natural decrease in viral infectivity over time, treatment with the FLOW technology resulted in a 50-fold reduction in infectious SARS-CoV-2 when compared to the untreated controls. Moreover, the majority of the viral inactivation occurred during the first 4 h of exposure to the FLOW unit. After 4 h, infectious virus levels decreased at a rate that was similar to that of the control, signifying natural decay in infectivity. These data show that **the FLOW unit inactivates 97.7% of infectious SARS-CoV-2 virus** on stainless steel surfaces after 4 to 6 h of exposure when high levels of virus are present.

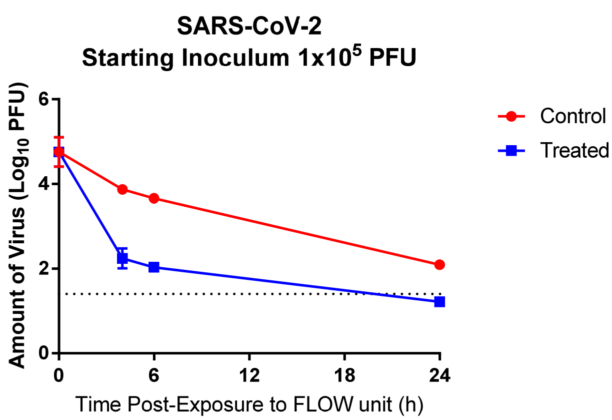

Figure 1. Recovery of infectious SARS-CoV-2 USA-WA1/2020 isolate inoculated onto stainless steel coupons at 10^5 PFU at various times after exposure to the FLOW technology (treated, blue squares and lines) or exposure to room temperature conditions (control, red circle and lines). Viral burden was determined by plaque assay on Vero E6 cells and the amount of virus was calculated by multiplying viral titer by 5 mls (the volume of the diluent media). Each data point corresponds to the geometric mean of three individual samples, error bars represent one standard deviation, and the dotted line signifies the assay limit of detection (1.4 $\log_{10}$ PFU).

This report is certified by Ashley Brown, Ph.D.

Ashley Brown

Ashley N. Brown, Ph.D.
Associate Professor
Institute for Therapeutic Innovation
Department of Medicine
College of Medicine

University of Florida
6550 Sanger Road
Orlando, FL 32827
Phone: (407) 313-7063