A Standardized Procedure for the Quantitative Evaluation of Residual Viral Activity on Antiviral Textiles

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Introduction: The SARS-CoV-2 pandemic has increased the demand for antiviral technologies to mitigate or prevent the risk of viral transmission. Currently, little is known about the role of textiles in cross-contamination and pathogen transmission despite the wealth of information on hard surfaces and fomites harboring viruses that remain viable in certain circumstances. Therefore, this pilot study aims to develop and refine a standardized protocol aimed at quantitatively evaluating residual viral activity on antiviral textiles. The $TCID_{50}$ method is frequently employed to quantitatively evaluate viral activity on textiles but has not been established as a standard. This procedure involves observing the cytopathic effect of a given virus on cells grown in a 96-well plate after seven days of incubation to determine the infectivity titre. We worked to improve the $TCID_{50}$ method through variations of different steps within the protocol to attain reproducible results. Once achieved, we intend to take a more comprehensive approach by analyzing the effect of multiple wash cycles and the construction (knit, woven, or non-woven) of reusable textiles on their long-term antiviral capacity. Our proposed standard method has the potential to provide evidence that supports further studies of textiles as a contributor to both the spread and mitigation of viruses.
Materials and Methods: Huh-7 cells and HCov-229E aliquots were generously supplied by the Scholle lab at NC State University and linen sheet samples were treated by the Goldshield company. The samples were laser cut into circles 9mm in diameter. Modified $TCID_{50}$ method was repeated in an attempt to optimize the protocol and developed the following procedure to assess antiviral activity on control and treated textiles. This also allowed for the investigation of textiles' capability to transmit microbes and infect human cells. Textile samples were placed in a new 96-well plate and viral suspension was added to the samples and eluted after 2 hours of infection time. The eluted volumes were serially diluted and added to 96-well plates confluent with Huh-7 cells. **Results:** Results are determined by observing the microscopic cytopathic effects (CPEs) caused by the virus in the monolayer of the cells. When cells were visually inspected, they were counted as positive if they showed CPE and negative if they were healthy. Cells undergo morphological changes when infected with virus and become flatter, clump together, and display a clear destruction of the host cell (Figure 1).

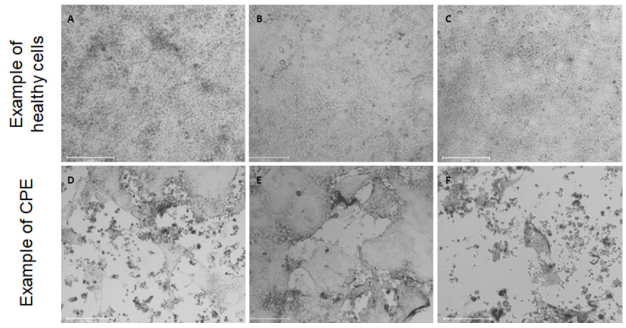

Figure 1: Examples of healthy cells and CPE. scale bar=275microns.

Panels A-C are healthy and do not exhibit CPE, thus identified as (-) for $TCID_{50}$ calculations. Panels D-F exhibit CPE and are identified as (+) for $TCID_{50}$ calculations. In terms of repeatability of the protocol and variation amongst different technicians performing the protocol, we achieved a very reproducible protocol. Examining the most recent repeats (about 10-15), we reached at least 99.5% confidence intervals within each test, indicating our technical skills to perform each test. To account for biological variation between different tests, as well as human variation due to different technicians performing the tests, we again achieved very low variance (confidence interval of 99.9%).
Conclusions: This developed protocol was prepared after careful performance and repetition of existing protocols after slightly varying the protocol between trials. In addition to creating a strong, repeatable, and effective protocol, we also discovered that the efficacy of an antiviral treatment can be visually determined by the difference between cytopathic effect and healthy cells. The next steps of this project will be to investigate the effects of laundry on the efficacy of the antiviral coating, i.e. temperature, type of detergent, and drying conditions. Additionally, the effects of light, temperature, humidity and other environmental conditions on the shelf life of the antiviral treatment will also provide a valuable input for the end use applications.