Assessment of Antiviral Material against SARS-CoV-2

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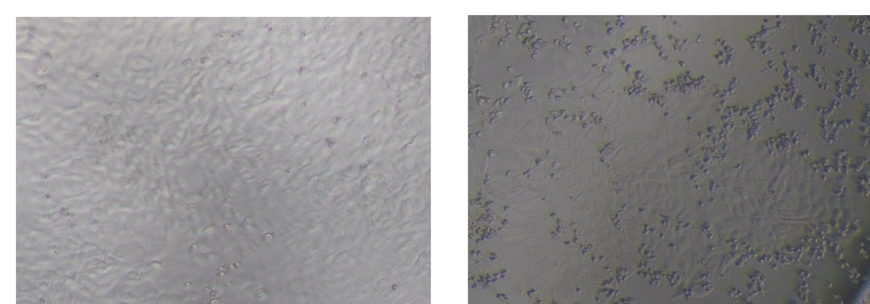
Introduction

The ongoing threat of COVID-19 infection and high rate of transmission, associated with severe illness, and fatalities, has created a severe threat to health care personnel, first responders, and general populations worldwide. This pandemic has brought about a need for an increased and rapid development of effective Personal Protective Equipment (“PPE”), and disinfection methods. In response to a client’s request, MRIGlobal conducted testing and evaluation of a novel antiviral fabric against SARS-CoV-2.

This testing utilized the Tissue Culture Infectious Dose ($TCID_{50}$) assay to determine if the treated fabric could successfully inactivate SARS-CoV-2 virus. Briefly, we inoculated control and test material with virus and incubated at room temperature for various time points. When time was up, samples were vortexed in DMEM/f12 culture media to resuspend any remaining virus. These samples were then diluted 1:10 (1 log per row) down a 96 well plate and added to Vero E6 host cells. The intention of this step is to determine how much the virus needs to be diluted before it is no longer able to infect the host cells. Plates were incubated and then read for cytopathic effects (CPE) after 4 days.



Healthy, non-infected cells Infected cells

CPE is read using a light microscope. Healthy Vero E6 cells are semitransparent with a fusiform appearance (pinched or narrowing ends and more round in the middle) in a monolayer of cells with little to no space between cells. Dead cells displaying CPE are often detached from the plate, round, less transparent, and much smaller than living cells. Any well displaying CPE is marked as positive whether the whole well is affected or only a small patch, as both are indicative of the presence of viable virus.

Materials and Methods

1. Cut control and test fabric to 2 × 2 inch pieces .
2. Inoculate samples with 200 µL USA-WA1/2020 virus stock (1.47E7 $TCID_{50}$/ml) by pipetting 25 µl from 4 tips of a multichannel pipette twice, for a total of 8 spots inoculated per coupon.
3. Incubate for determined time points.
4. Place fabric in conical tubes with 20 ml DMEM/f12 and vortex on high.
5. Dilute samples 1:10 down a 96 deep well plate in DMEM/f12.
6. Transfer dilutions to a plate of Vero E6 cells.
7. Incubate 35 minutes.
8. Add DMEM/f12 supplemented with 5% FBS.
9. Read plates for CPE.
10. Calculate $\log_{10}$ $TCID_{50}$/ml for control and test fabric using the Reed-Muench equation below.

$$\text{Proportionate Distance (PD)} = \frac{\%\text{CPE at dilution above } 50\% - 50\%}{\%\text{ CPE at next dilution above } 50 - \%\text{ CPE at next dilution below } 50}$$

$$TCID_{50} = 10^{\text{log of dilution above } 50\% \text{ CPE}} - PD$$

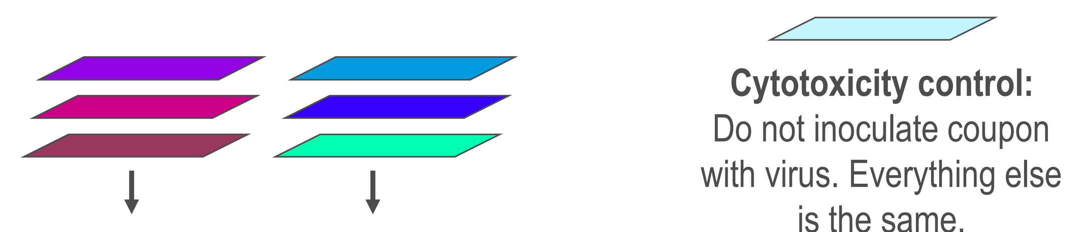
$$TCID_{50}/\text{ml} = \frac{1}{\text{volume used per well}} x \frac{1}{TCID_{50}}$$

$$\%\text{ Log Reduction} = 1 - 10^{-\text{ log reduction}}$$

$TCID_{50}$ Assay

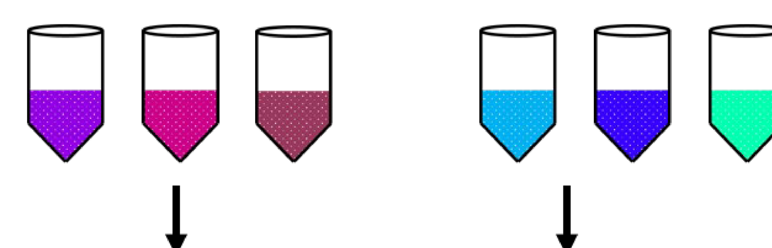
Set up n=3 coupons per test article/time point.

All coupons were inoculated with 200 µl virus. Test material coupons were collected at 0, 5, 30 and 120 minutes. Control coupons were collected at 0 and 120 minutes.

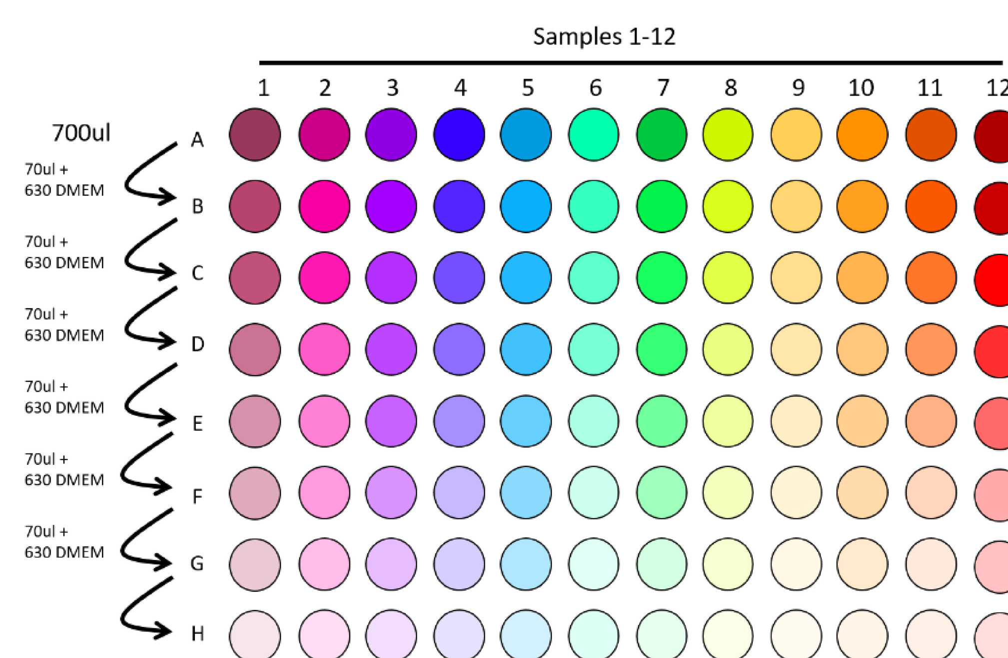


Each coupon was added to 10 ml DMEM/f12.

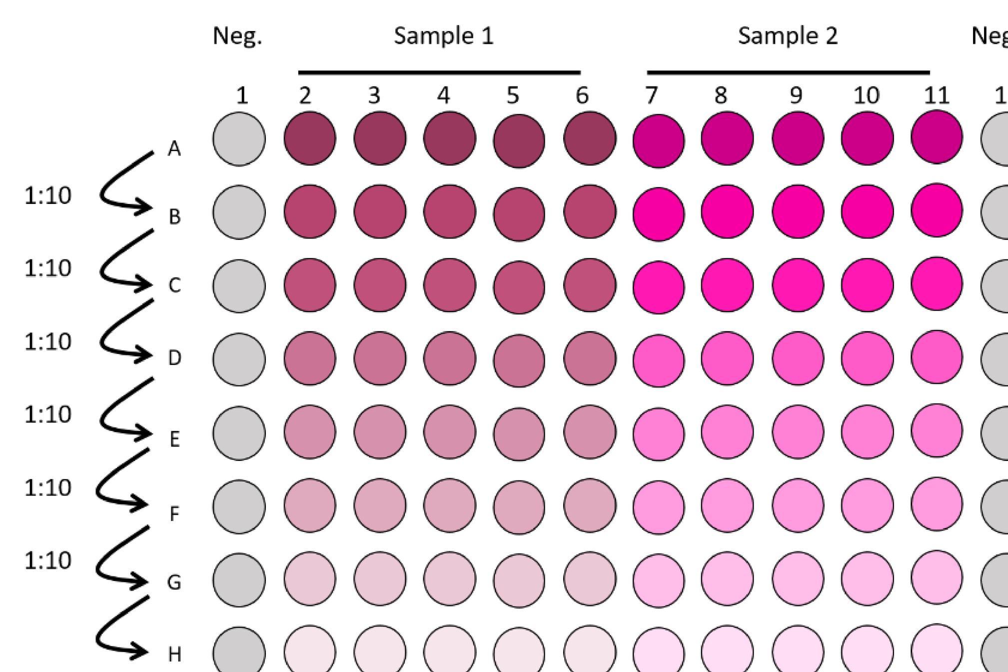
Coupons were vortexed on high 30 seconds to resuspend virus.



700 µl supernatant was transferred to a deep well plate. Samples were diluted 1:10 down the plate.



All dilutions were transferred to a 96 well plate of Vero E6 cells.



Plates were incubated 35 minutes, then DMEM + 5% FBS was added.

This process was done for all samples.

Results and Conclusions

Plates were read 4 days after the initiation of the experiment. For all contact times, test fabric solution reduced viral infectivity by 3.5 log (99.97%) compared to controls. The test $TCID_{50}$/ml values are the same as the test fabric cytotoxicity levels. This indicates that the test wells displaying cytotoxicity may not have had virus in them, it is just not possible to determine in this assay. Thus, the actual log reductions for test fabric compared to control fabric may be greater than reported here. The table below summarizes these findings.

Sample Name	Test Description	Contact Time (min)	Replicate	$TCID_{50}$/mL	Log10 $TCID_{50}$/mL	Average $TCID_{50}$/mL	Average Log10 $TCID_{50}$/mL	Log Reduction	Percent Log Reduction
T0-1	Test	0	1	≤ 3.16E+03	≤ 3.50	≤ 3.16E+03	≤ 3.50	≥ 3.52	≥ 99.97%
T0-2			2	≤ 3.16E+03	≤ 3.50				
T0-3			3	≤ 3.16E+03	≤ 3.50				
T5-1	Test	5	1	≤ 3.16E+03	≤ 3.50	≤ 3.16E+03	≤ 3.50	≥ 3.52	≥ 99.97%
T5-2			2	≤ 3.16E+03	≤ 3.50				
T5-3			3	≤ 3.16E+03	≤ 3.50				
T30-1	Test	30	1	≤ 3.16E+03	≤ 3.50	≤ 3.16E+03	≤ 3.50	≥ 3.52	≥ 99.97%
T30-2			2	≤ 3.16E+03	≤ 3.50				
T30-3			3	≤ 3.16E+03	≤ 3.50				
T120-1	Test	120	1	≤ 3.16E+03	≤ 3.50	≤ 3.16E+03	≤ 3.50	≥ 3.52	≥ 99.97%
T120-2			2	≤ 3.16E+03	≤ 3.50				
T120-3			3	≤ 3.16E+03	≤ 3.50				
C0-1	Control	0	1	1.47E+07	7.17	1.31E+07	7.02	N/A	N/A
C0-2			2	6.81E+06	6.83				
C0-3			3	3.16E+07	7.50				
C120-1	Control	120	1	1.47E+07	7.17			N/A	N/A
C120-2			2	6.81E+06	6.83				
C120-3			3	4.22E+06	6.63				
Control cytotox	cytotoxicity	N/A	1	3.16E+02	2.50	N/A			
Test cytotox	cytotoxicity		1	3.16E+03	3.50				
20210212KS-B CON	backtiter		1	1.47E+07	7.17				

Discussion

6.5-7 logs of virus were recovered from control fabric at both time points. This is approximately the same as the backtiter levels of the stock virus, which indicates that there is little loss of viral viability after 2 hours at room temperature. It also indicates that vortexing fabric in DMEM/f12 is an effective way to recover dried virus from a textile.

Compared to control material, test material returned no identifiable virus. However, the test material supernatant itself was cytotoxic to the host cells, killing the first row (1 log) of cells. Since it is impossible to tell if virus is present when the host cells are killed by anything other than virus, those wells must be marked as positive. However, this means it is possible that the actual inactivation capacity of the material is greater than what we were able to measure here. Overall, the test material proved very effective at inactivating virus, with maximum inactivation occurring almost instantaneously (t=0). Since virus is recovered in DMEM/f12, it cannot be ruled out that the inactivation is happening in the aqueous environment of the recovery step as opposed to on the material itself.

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