Antiviral capabilities of Curie Materials

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Executive summary

This report inspects the antiviral capabilities of the Curie innovation. First, it examines the arrest capability of the Curie Spunlace and Curie paper treated with 4% Curie Compound. Second, it inspects the Curie compound's cytotoxicity when introduced to the cells over 7 days. Third, it examines the Curie compound's antiviral activity against coronavirus (HCoV-229E) and enterovirus (Coxsackievirus B6). In the experiment addressing antiviral activity, infective viruses were first treated with Curie compound, and then diluted and mixed with living human or monkey cells to inspect over seven days if the virus has retained its capability to infect the cells after this treatment. Lastly, the report concludes the main results on antiviral properties of the product and its effects on uninfected cells based on the experiments performed.

RT-qPCR tests

The first test protocol is designed to detect the ability of the Curie Spunlace (Polyester+Cotton treated with Curie compound) and Curie Paper (Cotton treated with Curie compound) to eliminate the virus from the liquid. Control materials used for the test are regular tissue, FFP2 filter media, FFP2 non-woven meltblown.

Virus Strains:	Human coronavirus 229E (HCoV-229E), a virus belonging to the same family as SARS-CoV-2 that causes COVID-19, 50 000 PFU (infectious virus particles) per experiment. Coxsackievirus B6 (a member of enterovirus genus), 50 000 PFU (infectious virus particles) per experiment.
The test materials:	Curie Spunlace 4% (Polyester + Cellulose + Curie compound) Curie Paper 4% (Cellulose + Curie compound)
The control materials:	Regular tissue (used in laboratory) FFP2 filter media (Non-woven polyester) FFP2 sponge (Non-woven) Virus in buffer without any material (ultimate control)
The test protocol:	1. Cut a 2.5cm by 2.5cm piece of the material 2. Place the material into a 2ml Eppendorf tube 3. Apply 200µl of tissue culture supernatant containing 50 000 PFU of infectious virus particles on the test material and allow the virus containing buffer to soak into the test material 4. Incubate the test samples for 5 and 60 minutes at room temperature 5. Centrifuge the tube to remove the buffer from the test material 6. Remove the test material (filter materials and fabrics) from the tube 7. Test the virus concentration in the buffer recovered from the test materials (to detect RNA concentration in untreated virus and recovered buffer from the tests)

Test incubation times:	5 and 60 minutes
Parallel tests:	3 independent tests
Detection methods:	RT-PCR (test of virus RNA in the sample)

According to the protocol presented in figure 1, a 2.5*2.5cm piece of all tested materials was cut and analysed (each experiment was done in triplicates = repeated three times). Briefly, the material was first incubated in a buffer containing 50 000 virus particles; then, the tube was centrifuged to force all liquid out from the material tested. After centrifugation, the dry test material was removed from the tube, and 200µl of the buffer remained in the tube. This remaining buffer was analysed for the amounts of viruses using RT-qPCR, which detects viral RNA genome with high accuracy.

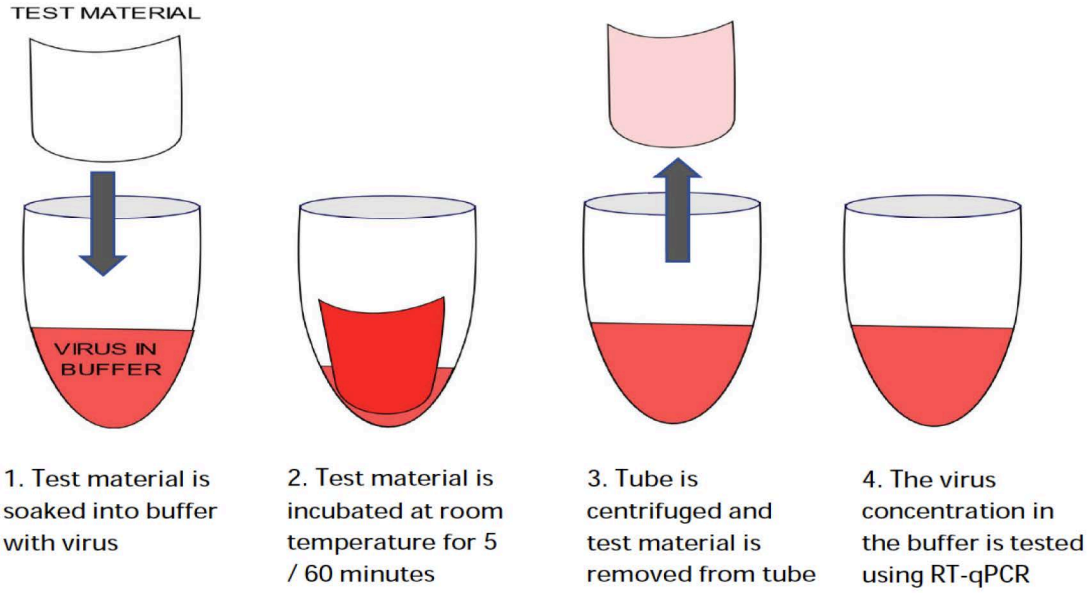


Figure 1 - The testing protocol to detect the antiviral activity of different materials tested

The experiments with the control materials (Tissue, FFP2 Filter media and FFP2 Sponge showed that the concentration of the coranavirus in the buffer remained high after the removal of the test material (Figure 2). Paralleling triplicate experiments provided similar results with low variance. The reduction in virus amounts ranged from about 50% to almost nothing suggesting that these control materials could not eliminate the virus from the buffer. This was true even after a long incubation period (60 minutes). The conclusion is that the control materials used in the study have no ability to eliminate the viruses from the buffer and have a minor affinity to the tested viruses. The experiments with the Curie Spunlace (Polyester + Cotton) and The Curie Paper materials showed that they had a strong ability to eliminate cornavirus from the buffer. In fact, with the model coronavirus, 2 out of the paralleling 3 repeats were completely virus negative. This means that the virus was below the detection limit of this sensitive RT-PCR assay (almost no viral RNA remained in the buffer). Thus, from the initial 50 000 virus particles that were initially introduced

into the buffer practically all were attached to the Curie material. Most likely, this was the result of the extremely strong affinity between Curie materials and human coronavirus 229E (Figure 2.)

The experiments with Curie material and a model enterovirus Coxsackievirus B6 (CVB6) showed very similar results - after 5 min incubation time 3/3 paralleling repeats were completely CVB6 negative for both Curie Paper and Spunlace. Moreover, only 1/3 tests using 60 min incubation time found CVB in the buffer and in this single case the amount of the detected virus was very low (less than 10 virus particles compared to the initial 50000 particles introduced into the buffer; Figure 3.)

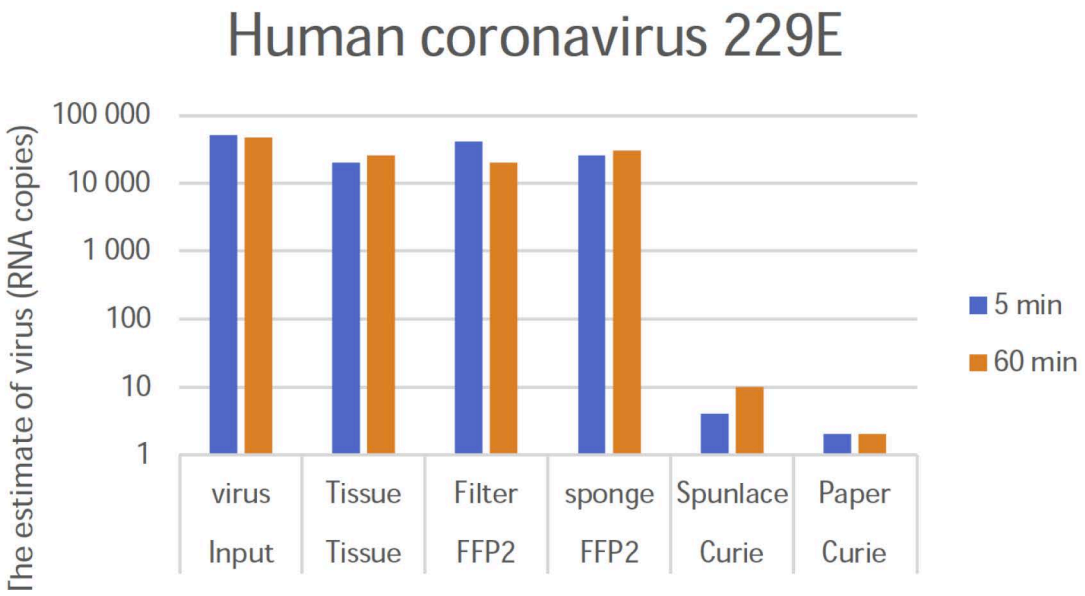


Figure 2 - Test results of the human coronavirus 229E - Virus RNA detected in the buffer using RT-qPCR, 2/3 for both curie materials were negative, only 1/3 of tests was able to find less than 10 active virus RNA copies in the buffer.

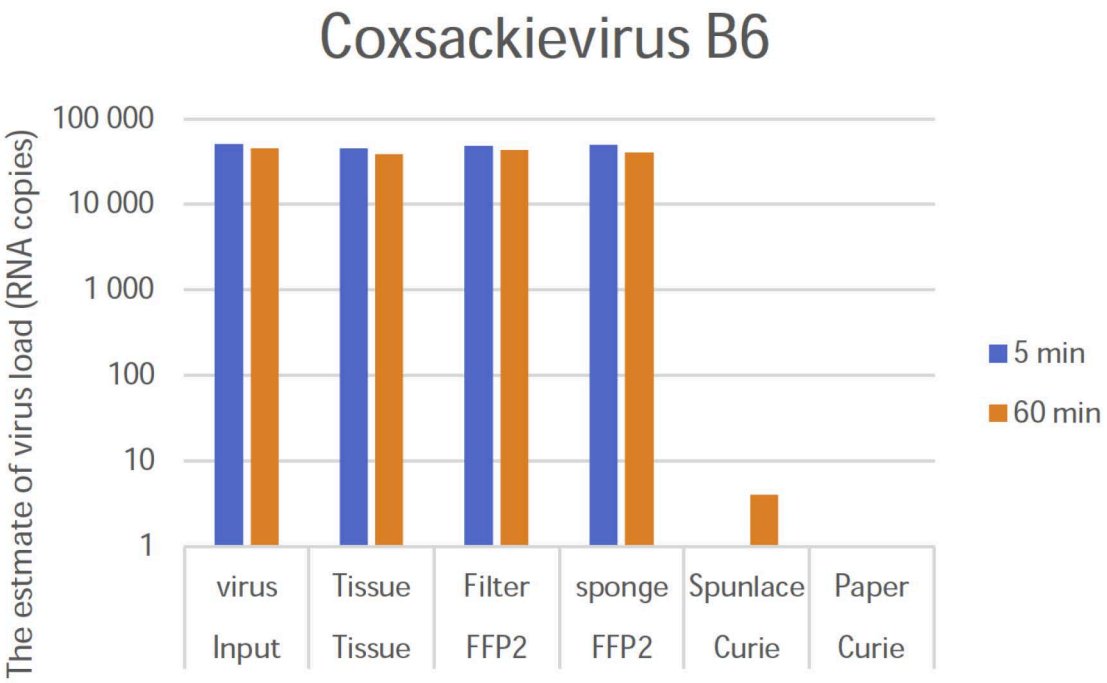


Figure 3 - Test results of the enterovirus (Coxsackievirus B6) - Virus RNA detected in buffer using RT-qPCR. For Curie Paper, 3/3 in 5min and 60min incubation times were entirely negative. At the same time, only 1/3 with Curie Spunlace had less than 10 viruses left.

The fact that similar effects were obtained using two viruses which have different structure (enterovirus and coronavirus) strongly suggests that the Curie Spunlace (Polyester + Cellulose + Curie compound) and the Curie paper (Paper + Curie compound) materials work well in the elimination of different types of viruses. These two viruses represented enveloped (coronavirus) and nonenveloped (enterovirus) viruses which means that their surface structure was very different. In addition, the observed 99.9% reduction of the viruses in the buffer was seen already after the shortest tested incubation time (5 minutes), suggesting a rapid effect of The Curie Spunlace (Polyester+Cotton+Curie compound) and The Curie paper (Paper + Curie compound) materials to the viruses, which is an essential feature to the mask material's functionality and suggests a high efficacy to segregate viruses.

Cytotoxicity experiments

These tests were designed to inspect the toxicity of the Curie Compound when introduced to the living GMK (Green Monkey Kidney) and MRC5 (Medical Research Council cell strain 5, which have originated from human lung tissue) cells. The test was designed to determine the Curie compound's concentration levels, which are not toxic to living cells of non-human primate and human origin. Cell culture media was used to dilute the liquid formulation of Curie compound. Each concentration was replicated in 8 paralleling wells in a 96-well format sterile cell culture plate. The compound was originally provided as a 30% solution. The first dilution of the compound was made to contain 16% active chemical in the volume of 200 microliters. The, 100 microliters of this 16% solution was mixed with 100 microliters of cell culture medium to generate 8%

concentration of the active compound, and this was further diluted using 2 folds serial dilutions. The liquid in each well was transferred into a culture plate and equal volumes of cells suspended in full media was added into each well. Cells were incubated at 37°C for 5 days until the bottom of the plate in each well became covered with cells that replicated during the 5-days' incubation time. Control wells named "Mock" contained cell culture media in the place of the active chemical. Cells were fixed and stained at the end of day 5 to visualise the cells which stay alive and managed to keep replicating and being attached to the plate. The wells which appear blue contain live cells while clear wells represent cytotoxicity.

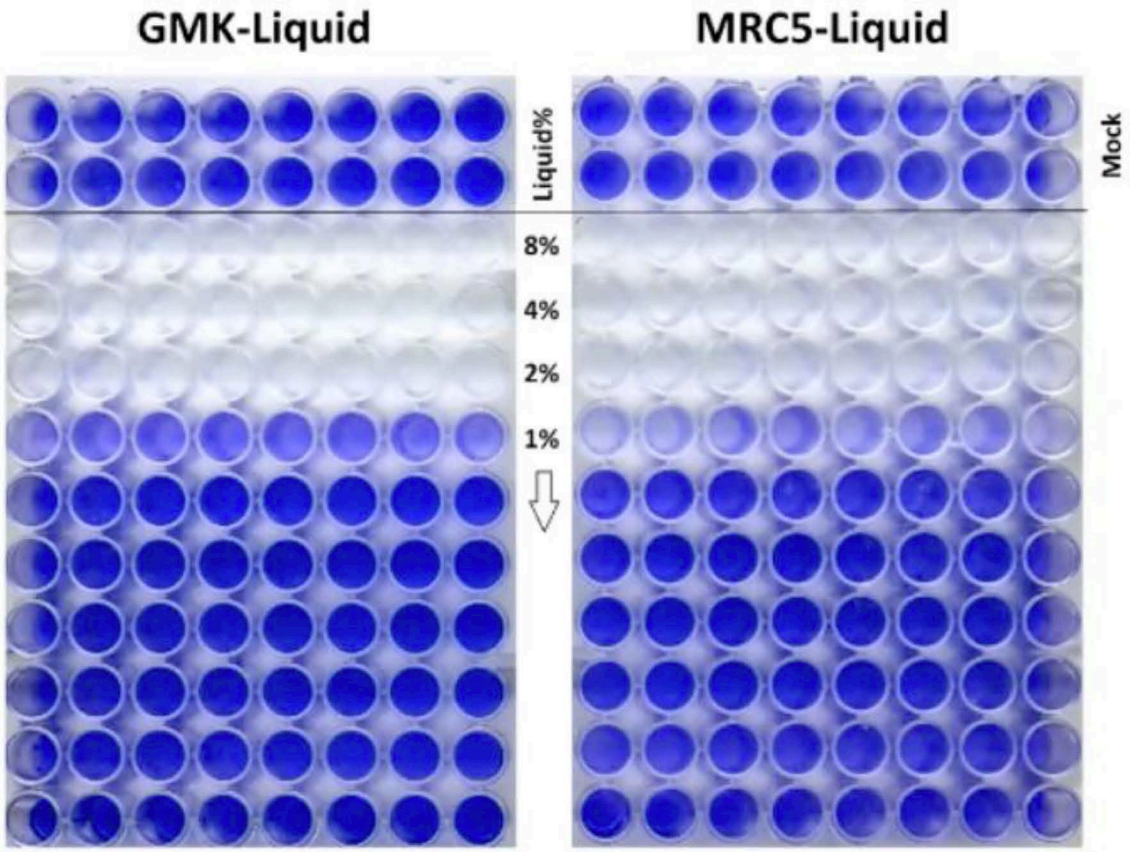


Figure 4 - Test results of the chronic cytotoxicity of Curie compound applied on the GMK and MRC5 cells in different concentrations. The first two lines are control lines (cells and cell media), and from top-down, a different concentration of Curie compound was applied to the plate. Cell media was used to dilute the mixture to 8%, 4%, 2%, 1%, 0,5%, 0,25%, 0,124% and so on. Blue color indicates living cells.

The Figure 4 represents the plates stained at the end of the experiment. As seen, the dilutions starting from 8% (the highest used in the assay) down to 1% interfered with the cells suggesting that they affected the viability of the cells. At 0.5% concentration of the chemical the wells remained comparable to control wells showing no visual cytotoxicity. The wells were also observed under microscope for possible presence of live cells which might have been replicating in suspension to rule out the possibility that the chemical would prevent their attachment to the plastic. Thus, the concentrations of 0.5% and less of Curie compound were found non-toxic for the cells over the period of 5 days constant exposure (Figure 4.). Results suggest that addition of the Curie chemical to the cell culture at a concentration of less than 0.5%, does not prevent cells from

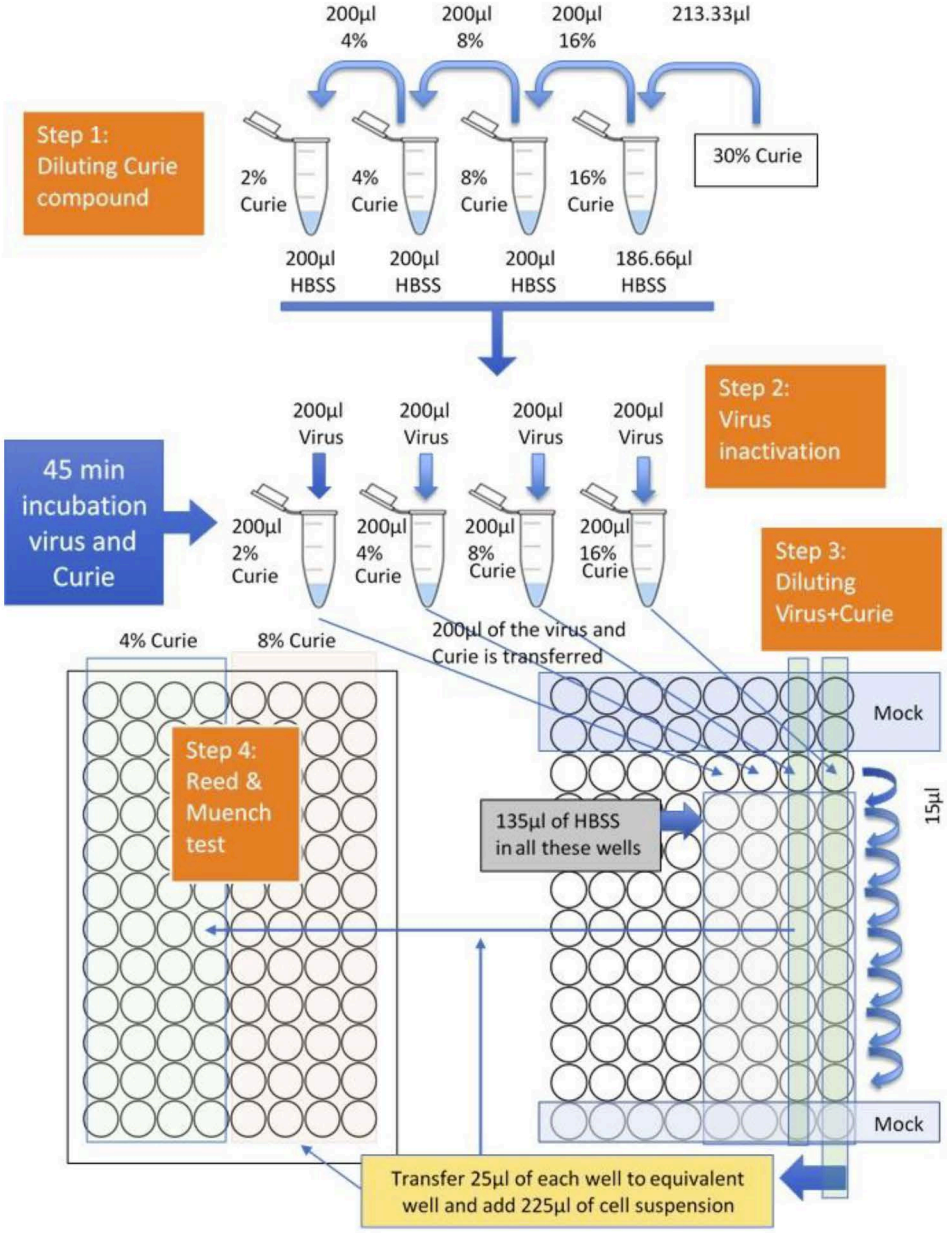
replicating and generating a full cell layer that is firmly attached at the bottom of the plastic plate. This suggests that at these concentrations Curie does not have any clear harmful effects on the cells. It should be noted that the concentrations calculated in the present experiments were based on the concentration of the stock chemical which was recorded on the tube (30%) and we did not independently measure the concentration of this original stock vial.

Antiviral capability of Curie liquid

Antiviral activity of the Curie compound was tested using enterovirus (Coxsackievirus B6) and coronavirus (human coronavirus 229E), which have different virion structure. Even though other virus families have not been included in this study, it is likely that the chemical could affect the infectivity of other viruses in a similar manner as it did in the case of the tested viruses in this study.

Virus Strains:	Human coronavirus 229E (HCoV-229E), family virus with SARS-CoV2 Coxsackievirus B6 (Enterovirus)
The test materials:	Curie Compound in different concentrations including 8%, 4%, 2% and 1% active material (defined based on the original vial having 30% active material delivered to our laboratory)
The control materials:	Control Mocks with no virus (two above lines and bottom line of the test plates) Input virus only
The test protocol:	1. Use Eppendorf tube and 96-well U-shaped plates for diluting the chemical and adding the virus 2. Dilute chemical in the Hank's balanced salt solution (HBSS) to 16%, 8%, 4%, and 2% in 2ml Eppendorf tubes 3. Add equal amounts of virus (4.00E+08 $TCID_{50}$/ml) and chemical at defined concentrations (final concentration of 8%-1%) to separate wells of 96-well plate to allow the chemical to inactivate the virus at the test concentration. 4. Add virus to HBSS with no chemical at equal volumes to represent the positive control for the virus infectivity 5. Mix well and incubate for 45 minutes 6. Serially dilute the mixture (at various concentrations of the chemical) with HBSS at 10-fold dilutions (1/10, 1/100) 7. Add 25 microliters of the mixture (chemical and the virus) to the test wells of the 96 well flat-bottom culture plates. 8. Add 225 microliters of the cell suspension containing approximately 40000 cells (GMK for CVB6 and MRC5 for coronavirus) per well 9. Dilute the virus at the same dilutions as the test wells

10. Incubate at 37°C for 5 days in humid chamber with 5% CO_2 and inspect the developments after 5 days



Test incubation times:	45 minutes to treat virus and to detect antiviral activity 5 days
Parallel tests:	4 independent tests for each dilution
Detection methods:	Optical detection of the developments in cell culture (Strong blue well = all cells alive and strong; bright well = all cells infected and dead)

Enterovirus

In this experiment the virus was coupled with Curie compound for 45 minutes at room temperature to allow the virus and compound to react. Then the virus and Curie mixture subjected to end point titration of the virus infectivity in a method called Reed and Muench. The experiment of determining the Curie compound's antiviral activity on enterovirus suggests that the Curie compound blocked the virus infectivity when diluted 100 times in all Curie concentrations tested (from 1% to 8%). Diluting the virus-chemical mixture after 45 minutes incubation at higher than 100 times removed antiviral effects. This showed that the original virus was blocked when it was absorbed into the chemical. As we can conclude from Figure 5, rows -1 and -2 formed a deep blue layer of cell culture, indicating that virus infectivity was blocked. In comparison, rows -1 and -2 with input virus are bright, indicating all cells are infected and dead since there were viruses, but no Curie blocking agent (Figure 5.) When the mixture was diluted more than 100 times the virus could be released from the mixture and was able to infect cells as seen in 100 dilutions downwards.

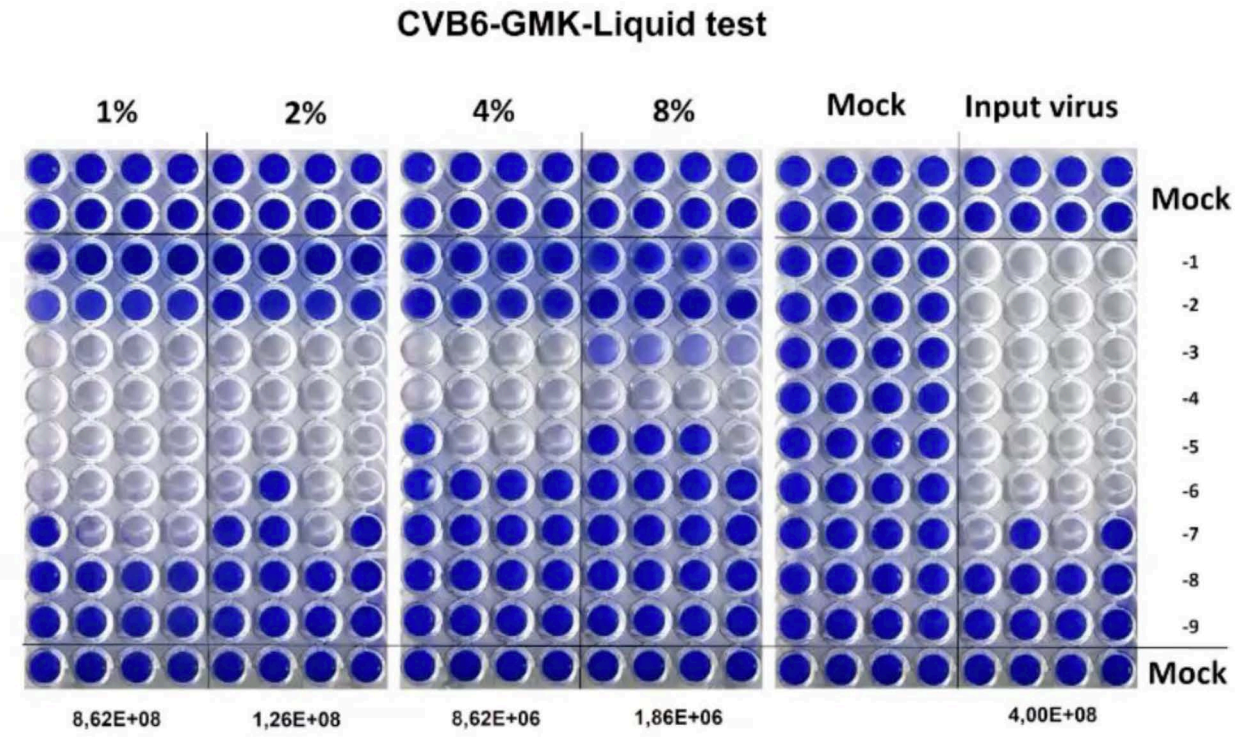


Figure 5 - Test results of the antiviral activity of Curie compound on enterovirus as tested in GMK cells.

Coronavirus

We tested the blocking effect of Curie compound for coronavirus the same way as CVB6. In this experiment again the virus was incubated with known concentrations of 8%, 4%, 2%, and 1% final concentration of Curie active compound for 45 minutes at room temperature and the mixture was tested for virus infectivity using the end point titration in a method called Reed and Muench. The experiment to test the antiviral activity of Curie compound on coronavirus was performed as drafted for CVB6. The results suggest a high antiviral activity when the virus was treated for 45 minutes with the compound. Diluting the virus-compound mixture 10 and 100-times showed complete blockage of virus infectivity when 8% and 4% concentrations of the compound were tested, and partial blockage with lower concentrations which were effective in 10 times diluted mixture but not in 100 time diluted mixture. The results suggest that the Curie compound possesses clear antiviral activity caused by a mechanism blocking the virus ability to infect the cells.

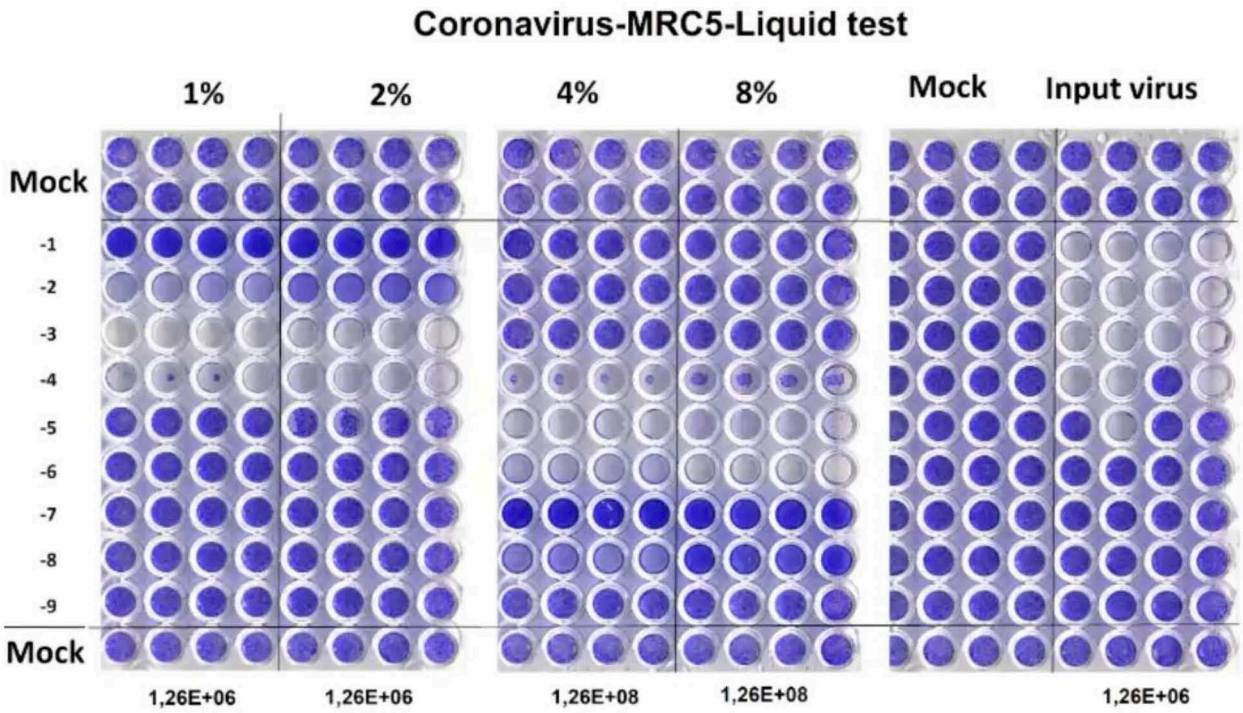


Figure 6 - Test results of the antiviral activity of Curie compound on Coronavirus as tested in MRC5 cells.

Conclusions

This report clearly concludes that Curie Spunlace (Polypropylene+Cellulose+Curie compound 4%) and Curie Paper (Cellulose+Curie compound 4%) have a clear ability to arrest coronavirus and

enterovirus with a 99.9% ratio. The result is significant considering that enterovirus and coronavirus are structurally different, suggesting that Curie Spunlace and Curie Paper are highly likely to achieve similar results with other viruses as well. Furthermore, the 99.9% reduction was seen already after the shortest tested incubation time (5 minutes), suggesting a rapid effect of the Curie Spunlace and the Curie Paper materials on viruses. Further on, the fact that Curie Compound showed no cytotoxicity in concentrations lower than 0.5% suggests that it may be used for the manufacturing of e.g. masks which are used externally under such conditions. Taken together, these findings showed no indications that Curie Spunlace and Curie Paper would have toxic effects when used under such conditions. Lastly, the study found that the Curie compound inhibited the ability of enteroviruses and coronaviruses to infect cells. We therefore further conclude that Curie compound is a promising substance for masks and other applications that aim at preventing virus spread.