

REPORT: MEASUREMENT OF ANTIVIRAL ACTIVITY AGAINST BOVINE CORONAVIRUS ON SURFACES COATED WITH VIREXBUSTER

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1 Materials and Methods

Biological Materials:

- PT-cells CCLV-RIE11 (Charité)
- Betacoronavirus 1 / bovine coronavirus (infectious cell culture supernatant, Charité)

All experiments were conducted in BSL-2 facilities at the Fraunhofer IZI.

Tests were carried out in two independent experiments with two replicates each.

Preparation:

DaXem provided the test materials shrink-wrapped in plastic bags, labelled VireXbuster (coated) and the corresponding reference (non-coated). Each bag contained four pieces with the size of 5 x 5 cm.

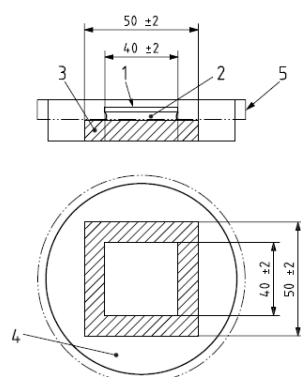
Bags were opened under sterile conditions. The coated side of the treated material was marked with a "c". Materials were disinfected by immersing them in 70% ethanol for five minutes. In the process, the silvery layer of the "coated" samples turned whitish.

Materials were kept in sterile petri dishes.

Experimental Procedure:

Day 1: Starting the experiment

1. Bovine coronavirus containing cell culture supernatant (5×10^9 TCID₅₀/ml) was brought to room temperature
2. VireXbuster treated (coated) and reference specimens (non-coated) were prepared in duplicates in sterile petri dishes with the treated side on top
3. 400 µl of the bovine coronavirus containing cell culture supernatant was pipetted onto the center of each test specimen, was covered with a 4 x 4 cm sterile OPP-foil
4. Foils were gently pressed down to ensure the evenly spreading of the test



inoculum

5. Petri dishes were placed in an air-tight sealed container
6. Additionally, 400 µl of the same infectious cell culture supernatant was pipetted into a test tube, serving as an internal control
7. A beaker filled with approx. 250 ml distilled H₂O was added to raise humidity

8. Incubation was carried out for 24 hours at room temperature, protected from light
9. PT cells were seeded in 96 well- plates (approx. 25 000 cells in 0,2 ml medium per well)

Results.....
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Day 2: Recovery of virus from test specimens and virus titration using TCID50

10. Petri dishes were opened after 24 hours of incubation time
11. OPP-foils were removed with a tweezer and placed reversed into the lid of the petri dish
12. The virus containing inoculum was recovered using 1 ml of culture medium (DMEM + 10 % fetal calf serum (FCS) and 1 % Penicillin/Streptomycin (P/S)), which was resuspended 4 to 5 times; the OPP-foil was also washed up 4 to 5 times with the same medium
13. The solution was transferred into a test tube
14. 1 ml of culture medium was added to the 400 µl internal control
15. Since a thin layer of fluid always remains on the surface of the material, the actual recovered volume was approx. 1,1 – 1,2 ml in average
16. For the TCID50 (tissue culture infection dose 50) assay, recovered samples were serially diluted (from 10^{-1} to 10^{-11}) in culture medium
17. The internal control A (Eppi 24 h) and a second control B (freshly thawed bovine coronavirus containing cell culture supernatant, untreated) were diluted in 10-fold serial dilutions from 1:100
18. Medium was removed from PT cells
19. 4 x 200 µl of each virus dilution was pipetted into the wells together with culture medium only as non-infected control
20. 96 well- plates were incubated for 4 days at 37 °C, 5% CO₂

Day 6: Fixation / staining with crystal violet

21. Cytopathic effect (CPE) was monitored in each well 4 dpi (days post-infection) microscopically
22. Cells were fixed with 70% ethanol (100 µl / well) for 20 min and stained with crystal violet (50 µl / well) for 10 min, plates were then rinsed with water and left to dry
23. Pictures of the plates were taken using a gel documentation system (Intas)
24. The TCID₅₀/ml of each recovered sample was calculated

2 Results

After 24 hours of incubation at room temperature on the surface of the VireXbuster-coated and non-coated (reference) test specimens, the bovine coronavirus containing cell culture supernatant was recovered followed by subsequent virus titration on PT cells to determine its concentration. The titer was measured with a TCID50 assay (figure 3). For each experiment, mean values of two replicates were calculated. Data of the two independent experiments were summarized by calculating mean values and standard deviations (table 1). Regarding the VireXbuster coated specimens, the following observation was made: a pH decline of culture medium was monitored in parts of the specimen which was visible by the change of the color from red to yellow (figure 1). This was potentially caused by a detachment of the coating material, which also resulted in a cytotoxic effect on the Vero E6 cells in the first dilution row of the TCID50 assay (figure

2). Since no cytotoxic effects were observed for the dilutions 10^{-2} to 10^{-11} , evaluation of the experiment was still possible.

Results.....

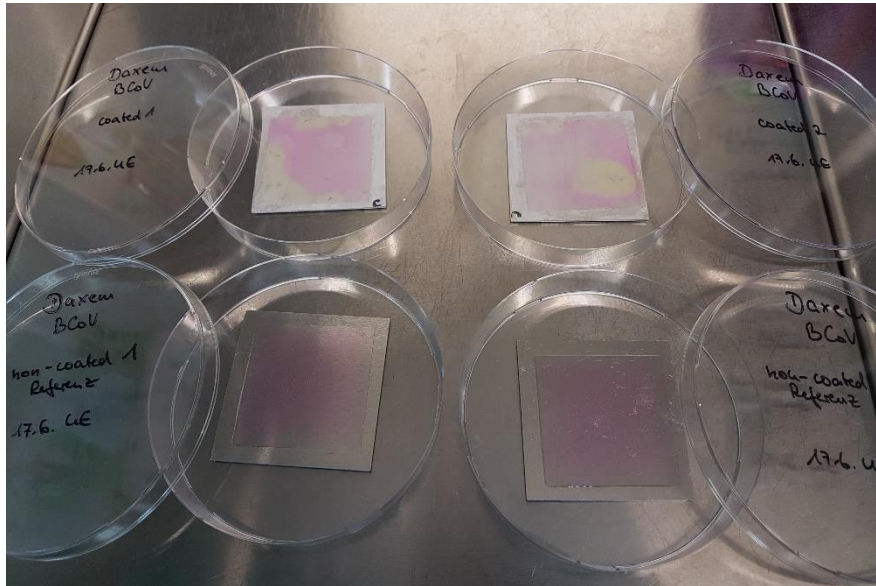


Figure 1: Picture of petri dishes from the first experiment after 24 hours incubation right before recovery. The two replicates of the coated (VireXbuster) test specimens are shown at the top. Yellow regions of the infectious cell culture supernatant under the OPP foil indicate a decrease of pH. This effect was not observed for non-coated reference as depicted at the bottom of the picture.

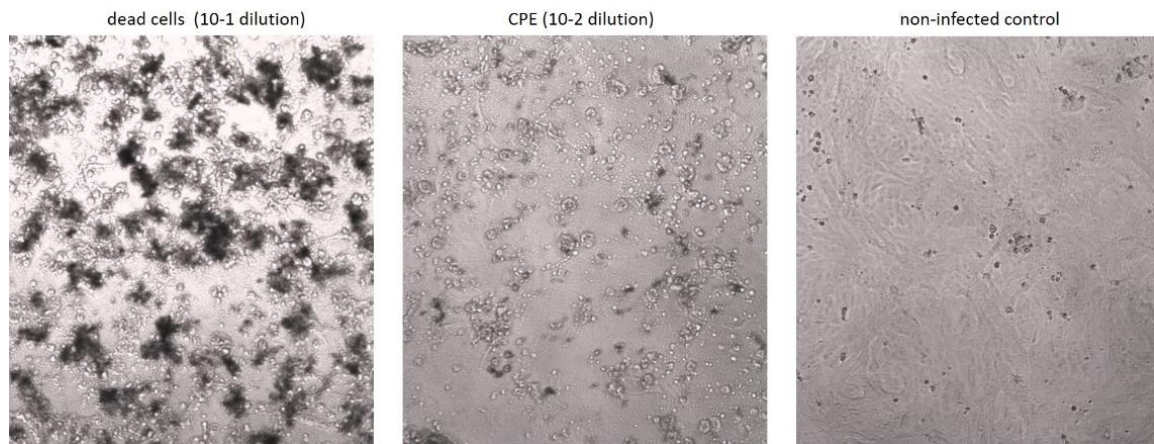


Figure 2: Pictures of selected wells before fixation taken with a microscope camera. First picture shows dead PT-cells, probably caused by cytotoxic effect of recovered bovine coronavirus containing cell culture supernatant due to a detached coating followed by pH decrease. Second picture shows CPE (cytopathic effect) caused by viral activity while third picture displays non-infected control with a confluent cell layer.

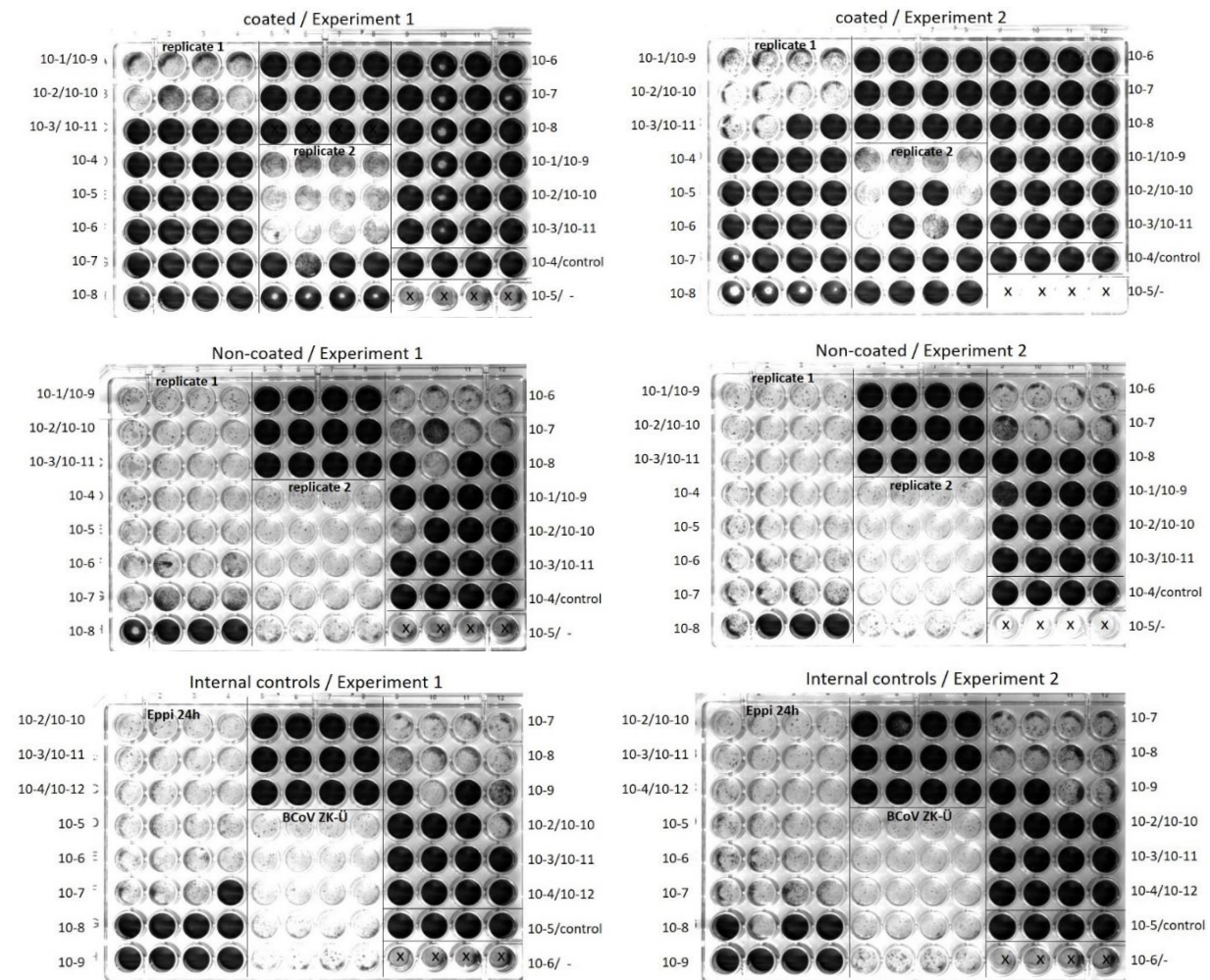


Figure 3: Pictures of TCID50 96 well -plates after fixation and staining with crystal violet, empty wells (white) indicate virus activity, while dark wells show confluent cell layers. Results of experiments 1 and 2 are shown, with 2 replicates per plate for coated (VireXbuster) test specimen and non-coated reference. The internal controls A (Eppi BCoV 24h) and B (untreated BCoV supernatant) are also shown.

Table 1: Titer of bovine coronavirus containing cell culture supernatant after recovery, summary of data, calculation of mean value from two independent experiments and standard deviation SD

summary	BCoV untreated	BCoV 24h RT	VireXbuster (coated)	Reference (non-coated)
experiment 1	3,00E+10	3,90E+08	1,98E+05	6,85E+08
experiment 2	1,80E+10	8,10E+08	2,85E+04	6,85E+08
Mean value	2,40E+10	6,00E+08	1,13E+05	6,85E+08
SD	8,49E+09	2,97E+08	1,20E+05	0,00E+00

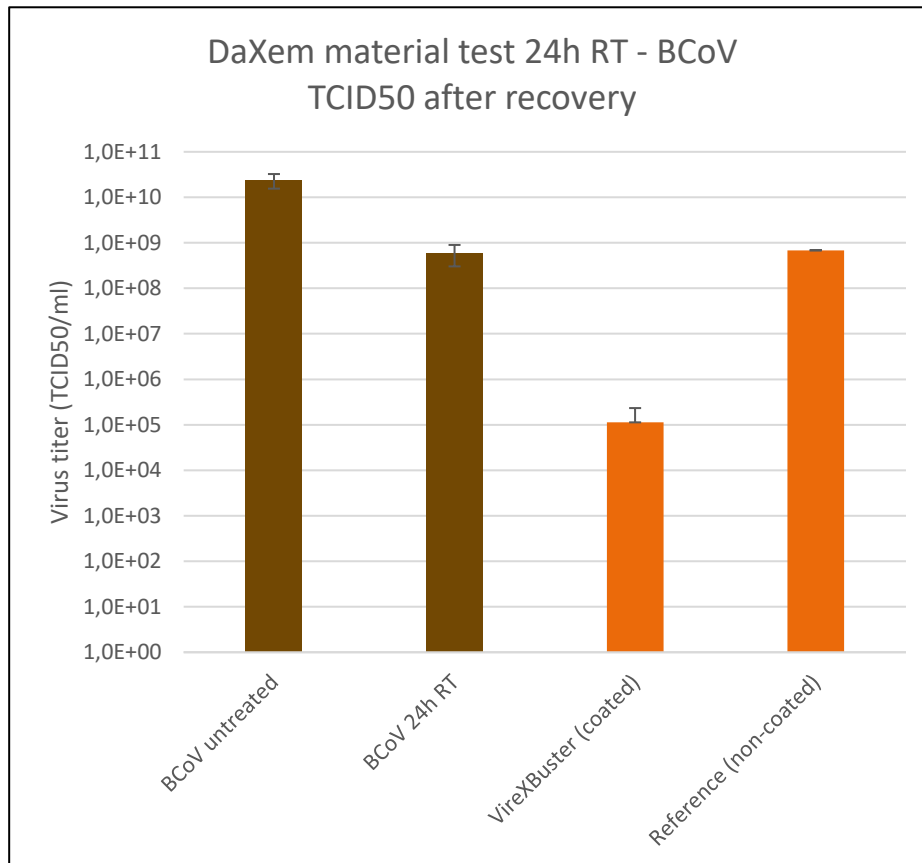


Figure 4: Titers [TCID50/ml] of bovine coronavirus containing cell culture supernatants after 24 hours incubation at room temperature on the surface of coated material (VireXBuster) and non-coated reference, mean values from two independent experiments and standard deviations are shown

3 Discussion

The VireXBuster coated specimens showed a significant virucidal activity against BCoV in comparison to the non-coated reference. A log titer reduction of 3,96 with a standard deviation of 0,595 was calculated, which means that the initial virus amount was reduced by more than 99,9%.

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(PD Dr. S. Ulbert)