PATHOLOGY
laboratory medicine
FACULTY OF MEDICINE
UNIVERSITY OF BRITISH COLUMBIA

LABORATORY REPORT

THE EVALUATION OF ANTI-CORONAVIRUS ACTIVITY OF FCB OIL

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Date performed: June 2020

EVALUATION OF ANTI-CORONAVIRUS ACTIVITY OF FCB OIL VAPOR:

Report of tests conducted in Virology Laboratory

(Department of Pathology & Laboratory Medicine UBC June 2020)

By Dr. Selvarani Vimalanathan & Dr. James Hudson

OBJECTIVES:

1) To determine the ability of the FCB oil vapor to inactivate human coronavirus
2) To determine that FCB oil vapor has no cytotoxic effect on cultured cells

SUMMARY AND CONCLUSIONS:

Tests demonstrated that the oil vapor from FCB oil is capable of inactivating more than 99% of the test samples of human Coronavirus HCOV strain 229E, following 30 minutes or more exposure, as determined by standard virus plaque assays in the human cell line MRC5.

In separate tests the cultured cells were exposed to FCB vapor, but were not adversely affected, and continued to grow, according to microscopic examination. This suggests that the vapor should be safe to apply externally to humans.

MATERIALS USED:

The virus used in the tests was the human Coronavirus HCOV strain 229E, which is frequently used experimentally as a surrogate for the SARS-COV2, the virus implicated in the COVID-19 pandemic.

The cell line used for growing stocks of the virus, and for assaying (titrating) the virus ($1x10^6$ PFU/mL), was the human cell line MRC5.

These cells were also used in the cytotoxicity tests.

METHODOLOGY:

Experiments were conducted according to our previous publication (Vimalanathan and Hudson 2014) on antiviral properties of plant oil vapors, with some modifications as follows: briefly, aliquots (10 µL) of virus (200 pfu) were individually added on to sterile glass slides, within the biosafety cabinet. Test oils 2 mL per well were carefully added to 6-well trays, the glass slides containing 10 ul virus (usually in triplicates) were replaced on 6-well trays and exposed to oil for 15, 30, 45 or 60 minutes at room temperature. Slides were removed again and each exposed virus aliquot was reconstituted in 1 mL of medium. All samples were then assayed for virus plaque formation in MRC5 cells as described before (Vimalanathan and Hudson 2014). Canola oil, which does not inhibit corona virus, was used as a negative control.

The assay plates were fixed and stained with crystal violet, which stains only the areas where intact cells were present. The reduction of viral titer was quantified by counting the virus plaques on each assay plate.

Mean values and standard deviations (SD) were calculated as indicated in the tables.

RESULTS & DISCUSSION:

Initially we determined that the combination of cells and virus strain selected was able to provide a satisfactory plaque assay method, such that viral plaques were readily detected against the background of stained cells and could be counted to yield quantitative results (as shown in Figure 1).

In a time course study, replicate samples of the virus were exposed to the oil vapor for varying periods of time, from 0 to 60 minutes, followed by assays to determine residual infectious virus, by the plaque assay technique. The results are shown in figures 2 & 3. Increasing time of exposure to the oil vapor resulted in decreasing infectivity of the virus, more than 90% by 30 minutes, although 60 minutes was required for complete inactivation of the virus.

This experiment was repeated, with the same result. Thus the virus could be completely inactivated by at least 60 min exposure to the FCB oil vapor.

In separate tests, the cell cultures, from which most of the media were removed to leave a moist monolayer of cells, were exposed to the vapor for various times, and periodically examined microscopically for cytotoxic effects. Following exposure, fresh media were added back to the cells to permit them to continue growing. In all cases, the cell cultures looked quite normal under the microscope, with no sign of cytotoxic effects.

Figures and legends are shown separately on succeeding pages

Reference:

Vimalanathan, Selvarani, and Hudson, James. “Anti-influenza virus activity of essential oils and vapors.” American Journal of Essential Oils and Natural Products, vol. 2, no. 1, 2014, pp. 47-53

DEFINITIONS OF TERMS USED:

Infectious. The infectivity of a virus refers to the ability of the virus to establish an infection in susceptible cells

Inactivation. This refers to viruses that have been rendered non-infectious and can no longer establish an infection.

Plaque assay. Focus of infection in cell cultures (see Fig 1 for illustration). Each plaque represents the multiplication of a single infectious virus

Cytotoxicity. Refers to damage or killing of cultured cells as a result of virus infection. May be observed under the microscope.

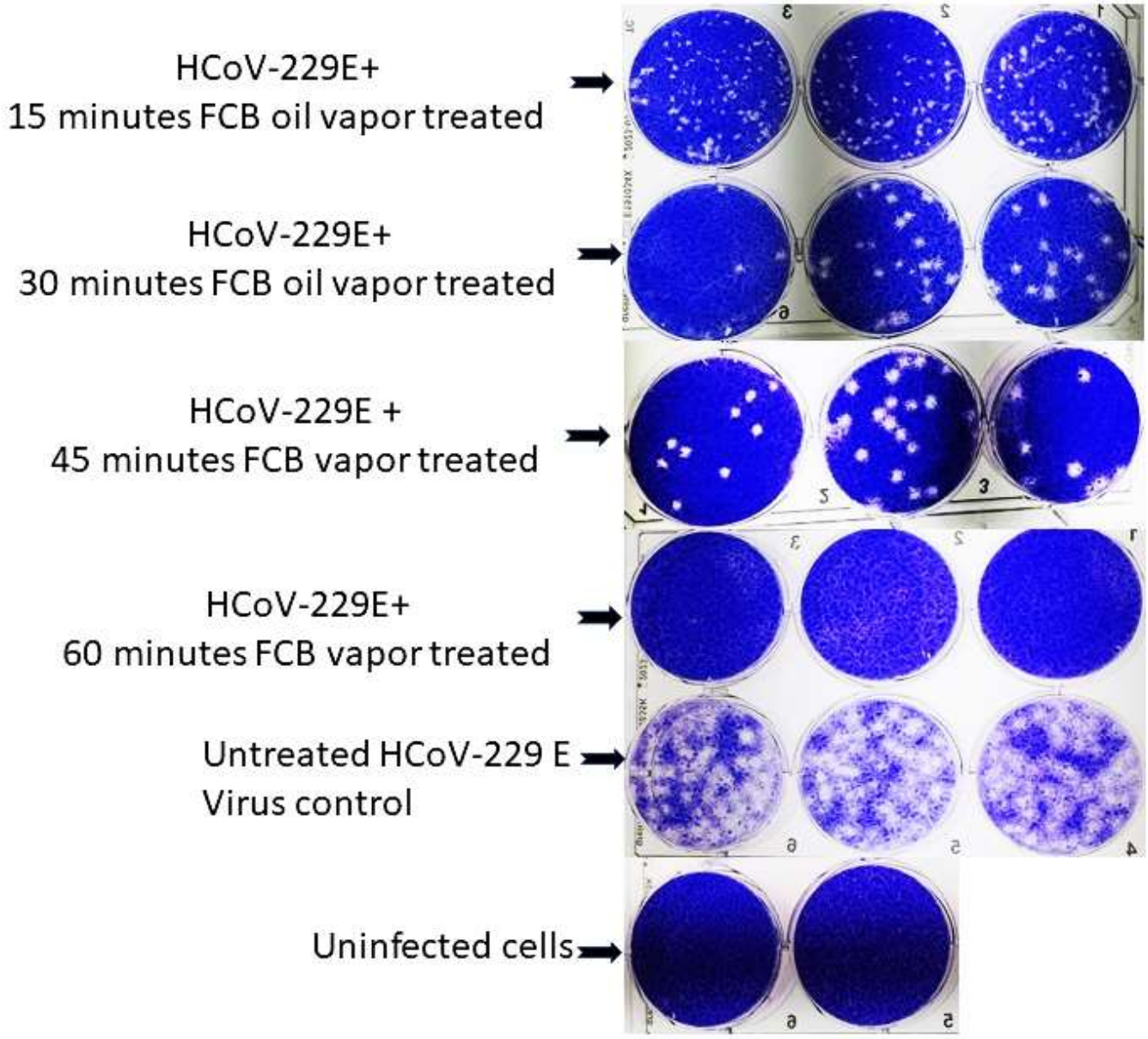

FIGURE 1. Results of one experiment showing the virus plaque assays
The blue/violet –stained areas show the presence of uninfected cells
The white unstained “holes” or “plaques” indicate the presence of areas where a virus has multiplied and killed the cells. Consequently, decreasing numbers of plaques indicates decreasing numbers of inactivated viruses

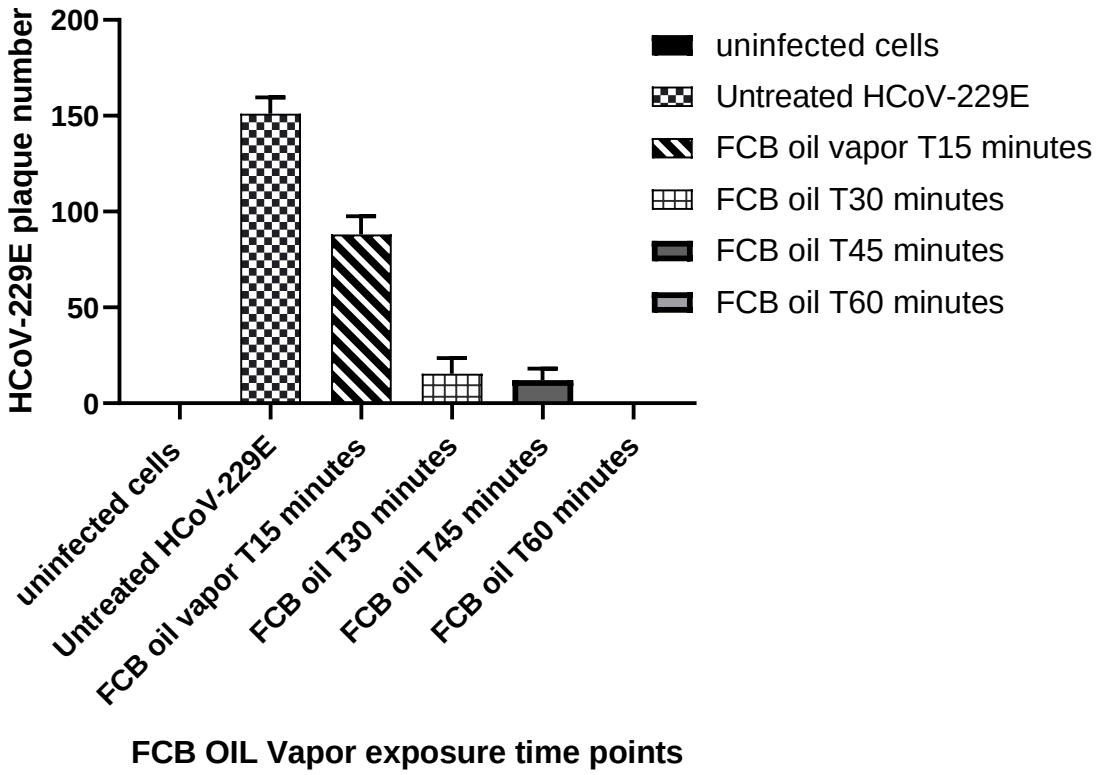

FIGURE 2.

Chart showing decrease in infectious virus with increasing exposure to vapor

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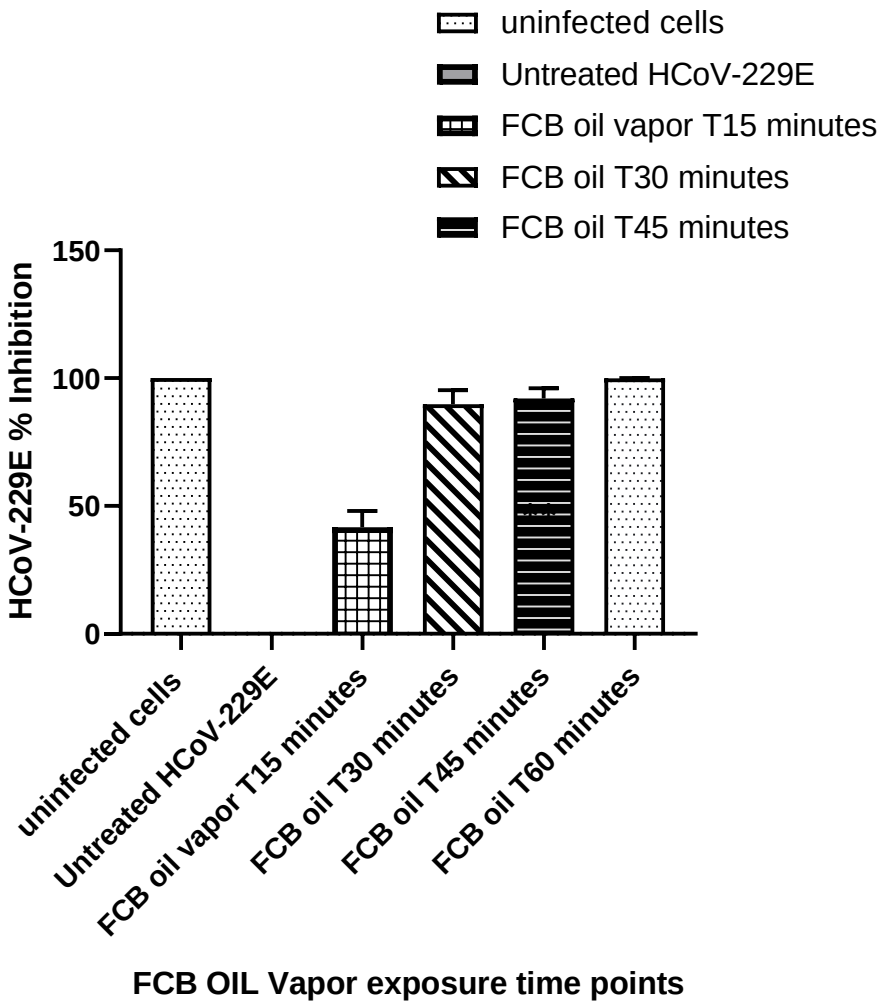


FIGURE 3.
Chart showing percent inhibition of infectious virus by increasing times of exposure to vapor

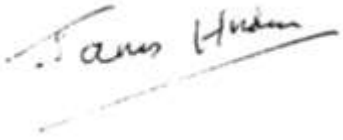
Table 1. Plaque reduction assay

	virus control HCoV-229 E			FCB oil vapor Treated		
	Mean	SD	N	Mean	SD	N
15 minutes	180	7	3	88	15	3
30 minutes	150	0	3	15	8	3
45 minutes	134	4	3	12	6	3
60 minutes	131	3	3	0	0	3

Table 2 % inhibition

Exposure time	% Inhibition	SD
15 minutes	42	6
30 minutes	90	4
45 minutes	93	5
60 minutes	99	5

TABLES 1 AND 2
Expression of data from Figures 1-3 in tabular format



EVALUATION OF ANTI-CORONAVIRUS ACTIVITY OF FCB OIL VAPOR:

By Dr. Selvarani Vimalanathan & Dr. James Hudson

EXECUTIVE SUMMARY

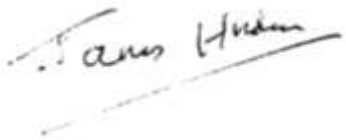
The objectives of this study were to determine whether FCB oil vapor could inactivate human Coronavirus, and to ensure that the vapor could not adversely affect human cultured cells.

The results showed that the FCB oil vapor was capable of completely inactivating the virus (more than 99.9 percent of its infectivity), based on virus plaque assays, without causing any adverse effects on the cells themselves.

This indicates that the FCB vapor could have a beneficial effect in humans infected by Coronaviruses.

Dr. James Hudson

Dr. Selvarani Vimalanathan



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