THE UNIVERSITY OF BRITISH COLUMBIA

FINAL STUDY REPORT

VIRUCIDAL PROPERTIES OF "NCCO-Invisible Glove Cream" WITH RESPECT TO SARS-CoV-2, Wuhan strain

Conducted for: RHT (North America) Industries Ltd.
100-1444 Alberni Street
Vancouver, BC, V6G2Z4, Canada

Conducted by:
Facility for Infectious Diseases and Epidemic Research (BCL 3, FINDER).
University of British Columbia,
2350 Health Sciences Mall,
Vancouver, British Columbia, V6T 1Z3, Canada

Author: Horacio Bach, Ph.D.

FINAL REPORT

Date: March 10, 2021
Report 02-2021

SUMMARY

This study was designed to evaluate the virucidal properties of "NCCO-Invisible Glove Cream" against SARS-CoV-2, Wuhan strain virus. In this test, the disinfectant reduced the virus titre by 3.63 logs after 5 minutes of exposure.

INTRODUCTION

A study was requested by RHT (North America) Industries Ltd. to evaluate a disinfectant product for its virucidal properties against SARS-CoV-2. The experimental work was conducted at the Facility for Infectious Diseases and Epidemic Research (BCL 3, FINDER). University of British Columbia, 2350 Health Sciences Mall, Vancouver, British Columbia, V6T 1Z3, Canada.

OBJECTIVE

To determine whether the disinfectant product “NCCO-Invisible Glove Cream” was virucidal against SARS-CoV-2, using accepted criteria for making virucidal claims.

MATERIALS AND METHODS

A. VIRUS STRAIN

The test virus used was the SARS-CoV-2 Wuhan strain used in this study was isolated at Sherbrooke hospital, Toronto, Ontario, Canada. The virus was stored at -80ºC before use.

B. CELL SUBSTRATE

The Vero E6 (ATCC CRL-1586) cells were obtained from ATCC. The Vero cells were stored in liquid nitrogen before use. Cells were thawed and sub-cultured in MEM media supplemented with 10% fetal bovine serum and 1 mM pyruvic acid and L-glutamine.

C. TEST PRODUCT

One container was supplied labeled “NCCO-Invisible Glove Cream”, Formula number: GL-01429A. DOM: July 31, 2020. Samples were received on 05/08/20. The sample was tested neat.

(See picture on next page)

D. REAGENT AND SUPPLIES

1. Medium MEM and DMEM x 2, supplied by Gibco.
2. L-glutamine, supplied by Gibco.
3. Fetal bovine serum (FBS), supplied by Gibco.
4. Trypsin, supplied by Gibco.
5. Flat-bottom microtitration plates, supplied by Corning.
6. Sterile Phosphate Buffer Solution (PBS), supplied by Gibco.
7. Methanol, Crystal Violet, para-formaldehyde, and cellulose supplied by Sigma.
8. OptiMem, supplied by Gibco.
9. Chromatography columns, supplied by BioRad.
10. Sephadex G-15, supplied by Pharmacia.

E. TEST CONDITION

Test Commencement Date	16/11/2020
Test Virus	SARS-CoV-2
Test Soil	N/A
Test Disinfectant	NCCO-Invisible Glove Cream
Test Temperature	22ºC
Exposure Time	5 minutes

F. PREPARATION OF SUBSTRATE

1. The preparation of the Vero cells was performed in a BCL 2 located at Jack Bell Research Centre, 411-2660 Oak Street, Vancouver, BC, V6H3Z6, Canada.
2. The Vero cell growth media was prepared by aseptically combining 10 ml of FBS to 100 ml of MEM supplemented with L-glutamine and pyruvic acid.
3. The contents of Vero cell flasks were aseptically aspirated using a pump. Cells were detached by adding approximately 2 ml of Trypsin.
4. Once the cells were lifted (~3 min), 40 ml of growth media was added, and the flask was shaken gently to suspend the cells in the media.
5. The flask contents were aseptically dispensed on a sterile 12-well plate at a final concentration of 1x 10^5 cells/well (confluency of 80%).
6. The plates were incubated in an incubator at 37ºC supplemented with 5% CO_2 for 18 hours.
7. The plates were taken to FINDER and used the same day of arrival, and maintained at the same culturing conditions.

G. CONDUCT OF THE POSITIVE VIRUS CONTROL AND TEST

1. SARS-CoV-2 was removed from the -80ºC freezer and thawed.
2. 10 µl of virus suspension containing a final concentration of 1x10^5 PFU was pipetted into a well (96-well format) and diluted with 90 µl OptiMem, representing 10^{-1} dilution. Serial dilutions were performed by taking 10 µl of the 10^{-1} dilution into 90 µl OptiMem, representing a dilution of 10^{-2}. This procedure was repeated until a dilution of 10^{-7} was reached.

H. INOCULATION OF THE TEST SAMPLES AND CONTROLS

1. The product was diluted with PBS (1:1, v/v) to facilitate the pipetting out of the container.
2. 10 µl of virus suspension (section G. 2) was mixed with 10 µl of the diluted material in a well (96-well format plate) and left for 5 minutes contact time.
3. Serial dilutions were performed as detailed in section G. 2. The 100 µl of each dilution was dispensed in the designated 12-well plates for 1 hour with gently manual mixing every 15 minutes at 37ºC. Then, the wells were overlayed with 1 ml of a mixture containing 0.5 ml sterilized cellulose 2% and 0.5 ml DMEM x 2 for 3 days. The plates were incubated in the CO_2 incubator with an atmosphere of 5%.

I. NEUTRALIZATION OF THE DISINFECTANT

1. Before performing the serial dilutions, the disinfectant was removed from the sample to avoid cytotoxic effects. 200 µl of a sterilized slurry of Sephadex G-15 resin in PBS (1:1 v/v) was placed inside a sterile chromatography column. The resin's flow-through was disposed and 100 µl of the sample containing the untreated or treated virus was collected by gravitation.
2. Serial dilutions of the virus were performed as detailed in G.2.

J. PLAQUE ASSAY

After 72 hours, the overlaying media was aspirated, and 2 ml of fresh paraformaldehyde was added to each well for 30 minutes. After removing the paraformaldehyde, 0.5 ml of an aqueous solution of 50% methanol containing 1% crystal violet was added to the wells for 10 minutes. Wells were gently washed with tap water until the cellular layer or the plaques were visualized.

K. RESULTS

The untreated SARS-CoV-2 control had a $\log_{10}$ titre of 4.8.

The virus used in the present study was inactivated by the tested product at a contact time of 5 minutes at room temperature (Table 1).

TABLE 1. LOG_{10} REDUCTION OF VIRUS AFTER TREATMENT

Treatment	**Titre (Log_{10})**	**Reduction (Log_{10})**
Virus Control	4.8	No reduction
5 minutes treatment	1.17	3.63

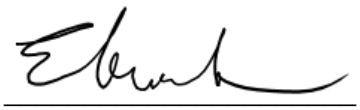
CONCLUSIONS

This study described herein clearly demonstrates that the tested product inactivated SARS-CoV-2 at room temperature with contact time 5 minutes.

(End of report)

AUTHENTICATION

I confirm that the study above was conducted by the staff of the Division of Infectious Diseases at the Immunity and Infection Research Centre, Vancouver Coastal Health Research Institute – The University of British Columbia.



Professor Ezra Kwok, PhD, PEng, MD, CCFP, CCAMA, FCFP, FEC
Chemical and Biological Engineering
Faculty of Applied Science, UBC
2360 East Mall
Vancouver, BC, Canada V6T 1Z3
Email: ezra.kwok@ubc.ca

Remark: A 3.63 Log_{10} viral reduction is equivalent to 99.98% viral inactivation.