

# Title: Innotech Nutrition Colflex Inactivation of Human Coronavirus 229e

## Protocol: Viral Inactivation Assays

Goal was to test 2 compounds at 4 contact times in triplicate for their ability to inactivate infectious Human Coronavirus 229e (HCoV229e).

HCoV229e was propagated and titered as previously published [1, 2].

The virus was mixed with "Soil"-5% Bovine Serum Albumin (BSA).

The infectious viral preparation, titered between a TCID<sub>50</sub> of 1x10<sup>5</sup> to 1X10<sup>6</sup>, was spread over the surface of the carrier-ceramic tile.

Colflex Oral Spray (C-Cinnamon) – Cinnamon-Lemon (*cinnamaldehyde, eugenol, linalool, thymol, carvacrol, limonene, carvone, B-Myrcene, and 1,8-cineole*)

Colflex Oral Spray (C-Mint) – Artic Mint (*thymol, carvacrol, limonene, carvone, B-Myrcene, and 1,8-cineole*)

Both Sprays were applied to the dried virus on the carrier by spraying three times the canister. C-Mint C-Cinnamon and C-Mint were left on the dried virus for contact times of 30 sec, 1 min, 2 min, and 5 min. At the end of each contact time C-Mint C-Cinnamon and C-Mint were neutralized and virus was removed and purified using Amicon filters as published [1]. Titering of remaining infectious virus was performed as previously published [1, 2].

Negative control was dried virus with no treatment to control for potential loss of virus due to drying. Positive control was treating the dried virus with 0.525% sodium hypochlorite for 30 sec.

Results of the study are shown in Table 1.

TABLE 1

TREATMENT	CONTACT TIME	TCID <sub>50</sub> 1	TCID <sub>50</sub> 2	TCID <sub>50</sub> 3	TCID <sub>50</sub> AVERAGE	Log <sub>10</sub> DECREASE	RANGE DECREASE
CONTROL	N/A	6.00	6.00	6.00	6.00	N/A	N/A
C-CINNAMON	30 sec	4.15	4.85	4.50	4.50	1.50	>90% - <99%
C-CINNAMON	1 min	4.00	4.00	3.75	3.92	2.08	>99 - <99.9%
C-CINNAMON	2 min	3.00	3.00	3.64	3.21	2.79	>99% - <99.9%
C-CINNAMON	5 min	3.69	4.00	4.36	4.02	2.00	≈99%
SODIUM HYPOCHLORITE	30 sec	0.00	0.00	0.00	0.00	5.83	>99.999%
CONTROL	N/A	5.75	6.00	5.75	5.83	N/A	N/A
C-MINT	30 sec	5.31	5.37	5.37	5.35	0.48	<90%
C-MINT	1 min	4.15	4.00	4.15	4.10	1.73	>90% - <99%
C-MINT	2 min	3.60	3.64	3.64	3.63	2.20	>99% - <99.9%
C-MINT	5 min	3.00	4.00	3.00	3.33	2.50	>99% - <99.9%
SODIUM HYPOCHLORITE	30 sec	0.00	0.00	0.00	0.00	5.83	>99.999%

Efficacy was greatest after at least 1 min of contact time. Contacts of 1-5 were generally between a 99% decrease in infectious virus and 99.9% decrease in infectious virus.

1. Meyers C, Kass R, Goldenberg D, Milici J, Alam S, Robison R. Ethanol and isopropanol inactivation of human coronavirus on hard surfaces. J Hosp Infect. 2021;107:45-9. Epub 2020/09/30. doi: 10.1016/j.jhin.2020.09.026. PubMed PMID: 32991941; PubMed Central PMCID: PMC7521917.
2. Meyers C, Robison R, Milici J, Alam S, Quillen D, Goldenberg D, et al. Rebuttal to overinterpretation of the antiviral results for human coronavirus 229E relative to severe acute respiratory syndrome coronavirus-2 by Rowpar Pharmaceuticals. J Med Virol. 2020. Epub 2020/12/30. doi: 10.1002/jmv.26762. PubMed PMID: 33372690.



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3/11/21  
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