

Experiment #1 Repeated with longer time of exposure and higher temperature

Part A. Effect of concentrated solutions of NaCl and boric acid on Great Lakes strain of VHSV for 11 days of contact at 15°C

Three replicate combinations (A, B, C) of **NaCl** at 100% (30g NaCl/100mL water), 50%, 25%, 0% with the Great Lakes VHSV strain (muskie MI'03) for **11 days of contact at 15° C**. Plaque assay performed on PEG treated EPC cells by inoculating virus dilutions of 1:10, 1:100, 1:1000, and 1:10,000 in duplicate wells of 24-well plate. Overlayed with methylcellulose after 30 min of virus adsorption, then incubated at 15°C for 7 days for plaque development, cells stained and fixed, plaques enumerated.

NaCl level log₁₀ pfu/mL

0% < 1.40

25% < 1.40

50% < 1.40

100% < 2.40 1st well toxic (note: pH was about 7 at full strength)

Results: Infectious virus was below detection limit for **all NaCl treatments** after 11 days of contact at 15°C.

Three replicate combinations (A, B, C) of **boric acid** at 100% (2.4g boric acid/100mL water), 50%, 25%, 0% with the Great Lakes VHSV strain (muskie MI'03) for **11 days of contact at 15°C**. Plaque assay performed on PEG treated EPC cells by inoculating virus dilutions of 1:10, 1:100, 1:1000, and 1:10,000 in duplicate wells of 24-well plate. Overlayed with methylcellulose after 30 min of virus adsorption, then incubated at 15°C for 7 days for plaque development, cells stained and fixed, plaques enumerated.

Boric acid level log₁₀ pfu/mL

0% < 1.40

25% < 1.40

50% < 1.40

100% < 2.40 1st well toxic (note: pH was 4.3 at full strength)

Results: Infectious virus was below detection limit for **all boric acid treatments** after 11 days of contact 15°C.

Part B. Effect of concentrated solutions of NaCl and boric acid on Great Lakes strain of VHSV for 2 h contact at 15°C and 37°C

Duplicate combinations (A, B) of NaCl (100%), boric acid (100%), or untreated (0%) with the Great Lakes VHSV strain (muskie MI'03) for **2 hr of contact at 15°C or 37°C**. Plaque assay performed on PEG treated EPC cells by inoculating virus dilutions of 1:10, 1:100, 1:1000, and 1:10,000 in duplicate wells of 24-well plate. Overlayed with methylcellulose after 30 min of virus adsorption, then incubated at 15°C for 7 days for plaque development, cells stained and fixed, plaques enumerated.

Temperature NaCl Boric acid Untreated

15°C 5.01 4.80 5.38
37°C < 2.40 2.70 2.70

Results: Compared to treatments at 15°C, the VHSV titer was reduced substantially by the 2 hr contact at 37°C even without the presence of NaCl or boric acid (2.68 log₁₀ reduction or 99.8%).

Summary: While short exposures (e.g. 20 min) showed little inactivation of VHSV in initial experiments, contact of the virus for: (1) a prolonged time (e.g. 11 days at 15°C) with only low levels of protein (0.1% fetal bovine serum) with or without additives (NaCl or boric acid) or (2) shorter duration (2 hr) at 37°C gave a reduction in virus titer of 99% or greater.