Efficacy of Common Disinfectant/Cleaning Agents in Inactivating Murine Norovirus as a Surrogate for Human Norovirus

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Outline

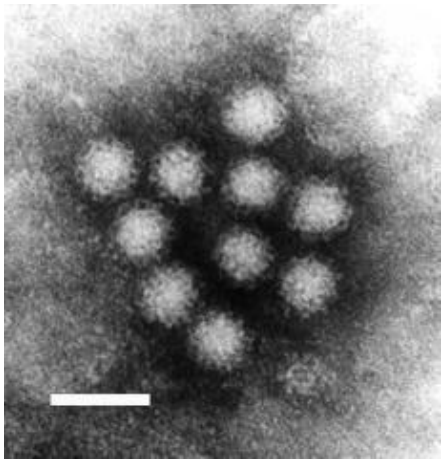
- **Background Information on Norovirus**
- **Objectives**
- **Methodology**
- **Results**
- **Summary**

Background

- Acute gastroenteritis
  - Inflammation of the stomach and small intestine
- Predominant cause of gastrointestinal infections worldwide
- Accounts for two-thirds of foodborne infections and is leading cause of worldwide epidemic gastroenteritis
- Common in BC

What is Norovirus?

- Single-stranded (+)sense RNA virus
- Caliciviridae family
- Small, round structured virus
- 35-40 nm in diameter



Source: F. P. Williams, US EPA

Lopman, B. A., Reacher, M. H., Vipond, I. B., Sarangi, J., & Brown, D. W. G. (2004). Clinical manifestation of norovirus gastroenteritis in health care settings. *Clinical Infectious Diseases*, 39, 318-324.

Zingg, W., Colombo, C., Jucker, T., Bossart, W., & Ruef, C. (2005). Impact of an outbreak of norovirus infection on hospital resources. *Infection Control and Hospital Epidemiology*, 26 (3), 263-267.

Norovirus

- 12-24 hour incubation period
- Symptoms 15-48 hours after exposure
- Infection lasts for 12-60 hours
- Main symptoms
  - Nausea, vomiting (Predominates)
  - Diarrhea
  - Abdominal pain
- Self-limiting infection

Hutson, A. M., Atmar, R. L., & Estes, M. K. (2004). Norovirus disease: Changing epidemiology and host susceptibility factors. *Trends in Microbiology*, 12 (6), 279-287.

Nuermberger, E. (2005). Current issues in the diagnosis, evaluation, and management of gastrointestinal infections. *Gastroenterology*, 5 (2), 90-97.

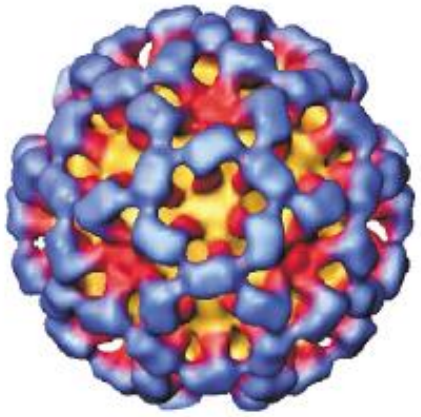
Transmission

- Fecal-oral route
  - 10-100 virion particles to become infected
- Person-to-person spread
  - Aerosol formation – projectile vomiting
  - Up to 3×10^7 virus particles distributed as an aerosol
- Foodborne transmission
  - Oysters, other food
- Waterborne transmission
- Inanimate environments
  - Surfaces (e.g. taps, handles, sinks)
  - Medical equipment

Nuermberger, E. (2005). Current issues in the diagnosis, evaluation, and management of gastrointestinal infections. *Gastroenterology*, 5 (2), 90-97.

Problems with Norovirus

- Virus has no lipid envelope and is composed of a robust capsid of a single protein
- Very resistant to:
  - Environmental degradation
  - Temperature
  - Desiccation
  - Chemical disinfection



Source: Hutson et al., 2004

Hutson, A.M., Atmar, R.L., & Estes, M.K. (2004). Norovirus disease: changing epidemiology and host susceptibility factors. *Trends in Microbiology*, 12 (6), 279-287.

Nuermberger, E. (2005). Current issues in the diagnosis, evaluation, and management of gastrointestinal infections. *Gastroenterology*, 5 (2), 90-97.

Wu, H. M., Fornek, M., Schwab, K. J., Chapin, A. R., Gibson, K., Schwab, E., Spencer, C., & Henning, K. (2005). A norovirus outbreak at a long-term care facility: The role of environmental surface contamination. *Infection Control and Hospital Epidemiology*, 26, 802-810.

Problems with Norovirus

- Obstacles for norovirus vaccine
- No anti-virals available
- Antigenic heterogeneity within family
- Immunity against norovirus short-lived due to variability and growing list of strains

Lindesmith, L., Moe, C., LePendu, J., Frelinger, J. A., Treanor, J., & Baric, R. S. (2005). Cellular and humoral immunity following Snow Mountain Virus challenge. *Journal of Virology*, 79 (5), 2900-2909.

Goodridge, L., Goodridge, C., Wu, J., Griffiths, M., & Pawliszyn, J. (2004). Isoelectric point determination of Norovirus virus-like particles by capillary isoelectric focusing with whole column imaging detection. *Analytical Chemistry*, 76 (1), 48-52.

Norovirus Outbreaks

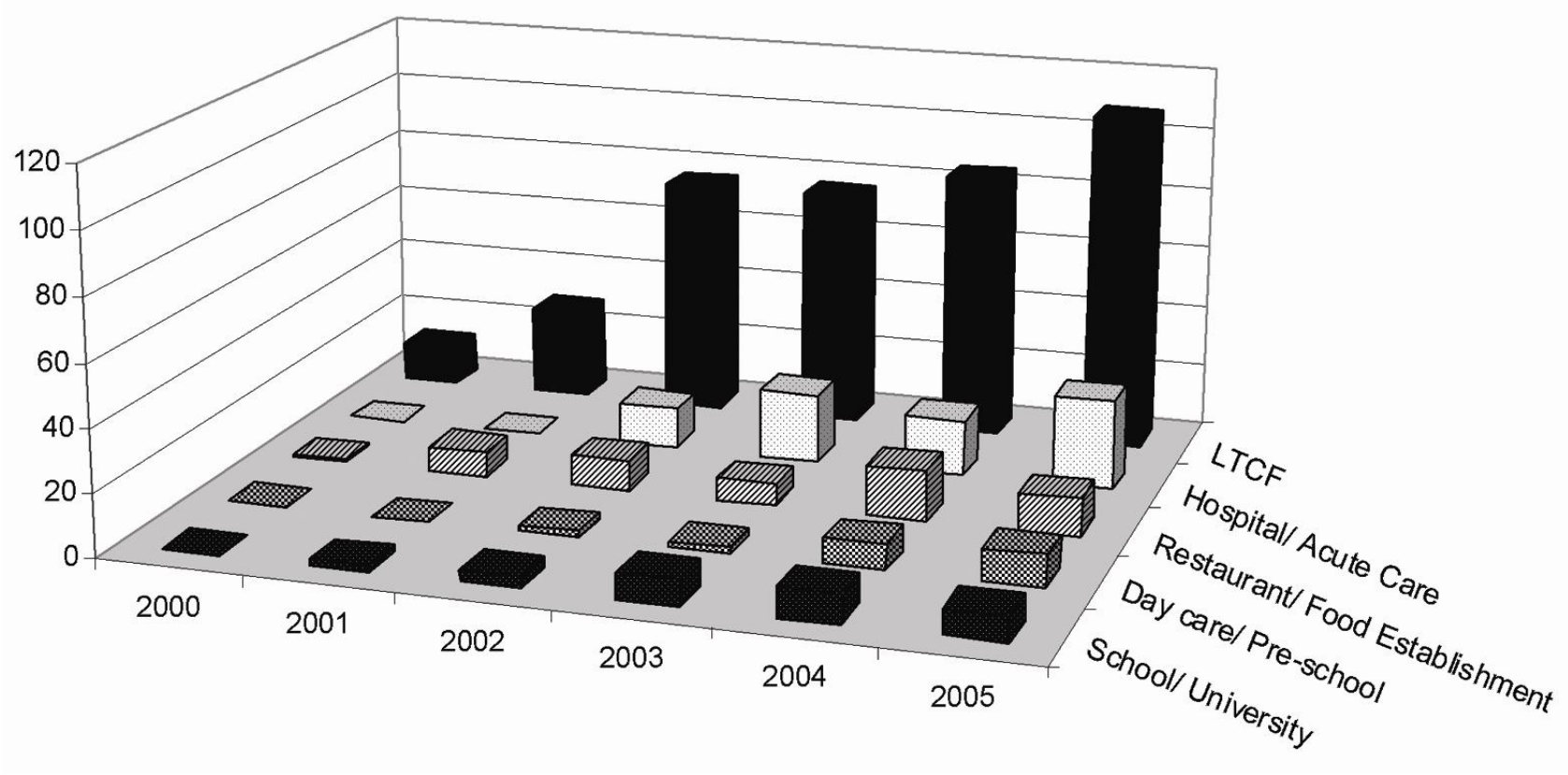
- Spreads readily and causes outbreaks in settings such as hospitals, daycare centres, residential care homes and cruise ships
- Issues with controlling and cleaning
  - Outbreaks in cruise ships occurring in consecutive cruises despite attempts to disinfect and sanitize the ships
  - Discontinuation of ships – vigorous cleaning and sanitization to stop outbreaks

Hota, B. (2004). Contamination, disinfection, and cross-colonization: Are hospital surfaces reservoirs for nosocomial infection? *Clinical Infectious Diseases*, 39, 1182-1189.

Norovirus Outbreaks

Norovirus Outbreaks in BC, 2000-2005

Source: Environmental Microbiology Laboratory, BCCDC Public Health Labs



Disinfectants

- Murine norovirus (MNV-1) was inactivated at 2600 ppm sodium hypochlorite with >4 log reduction at 0.5, 1 and 3 minute contact time
- Found quaternary ammonium-based disinfectants to be ineffective with <1 log reduction after 10 minutes
- Noro-like viruses such as feline calicivirus (FCV) are sensitive to ethanol, 1-propanol, isopropanol
- FCV inactivated in presence of sodium hypochlorite, chlorine dioxide, iodine or glutaraldehyde

Belliot, G., Lavaux, A., Souihel, D., Agnello, D., & Pothier, P. (2008). Use of murine norovirus as a surrogate to evaluate resistance of human norovirus to disinfectants. *Applied and Environmental Microbiology*, 74 (10), 3315-3318.

Girard, M., Ngazoa, S., Mattison, K., & Jean, J. (2010). Attachment of noroviruses to stainless steel and their inactivation, using household disinfectants. *Journal of Food Protection*, 73 (2), 400-404.

CDC Recommendation

- Recommends the use of chlorine bleach at a minimum concentration of 1000 ppm or other U.S. Environmental Protection Agency (EPA) approved disinfectants for controlling Norovirus outbreaks
- Areas with high level of soiling can use up to 5000 ppm of chlorine bleach

Surrogate Viruses

- Human norovirus has yet to be grown in cell culture
- This makes it difficult to assess the efficacy of disinfectants and cleaning agents
- MNV-1 and FCV have been used as surrogate viruses for human norovirus
- Suitability of FCV as a model should be used as caution
- Murine and human noroviruses are closely related

Poschetto, L. F., Ike, A., Papp, T., Mohn, U., Böhm, R., & Marschang, R. E. (2007). Comparison of the sensitivities of noroviruses and feline calicivirus to chemical disinfection under field-like conditions. *Applied and Environmental Microbiology*, 73 (17), 5494-5500.

Objectives

- Determine the efficacy of commonly used types of disinfectants and/or cleaning agents used in health care facilities in BC using murine norovirus as a surrogate for human norovirus
  - Murine norovirus (MNV-1)
    - RAW 264.7 macrophage mouse cells (ATCC TIB-71)

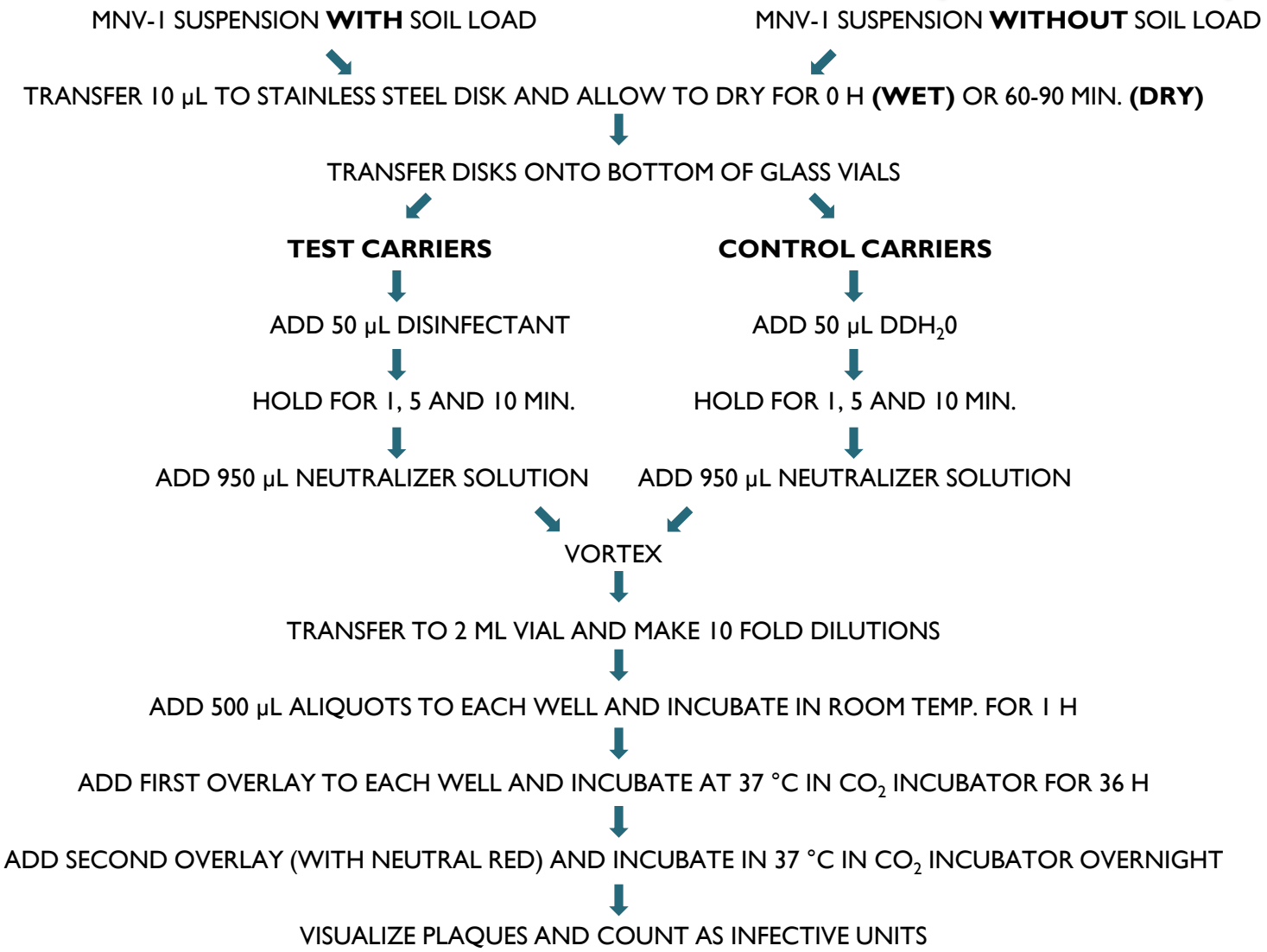
Methodology

- Springthorpe, V. S., & Sattar, S. A. (2003). *Quantitative carrier tests (QCT) to assess the germicidal activities of chemicals: rationales and procedures*. Ottawa, ON: Centre for Research on Environmental Microbiology.

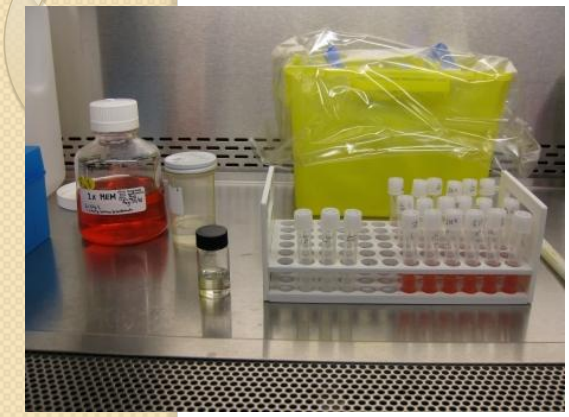
Disinfectants Used

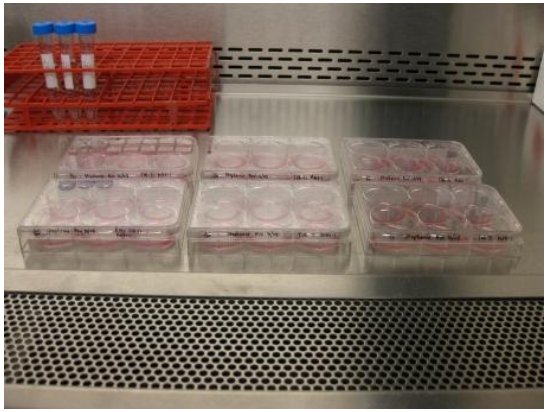
- Sodium hypochlorite – 5.4 %
- RTU quaternary ammonium – 0.28 %
- RTU accelerated hydrogen peroxide – 0.5 %
- Concentrated accelerated hydrogen peroxide – 7.0 %

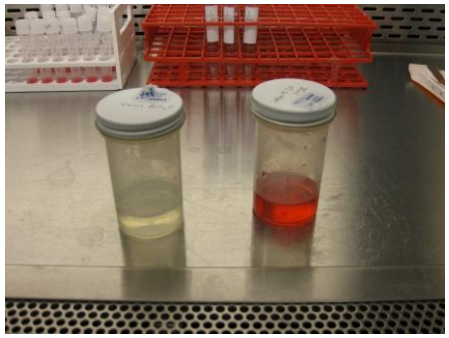
Flowchart of MNV-1 (QCT-2)

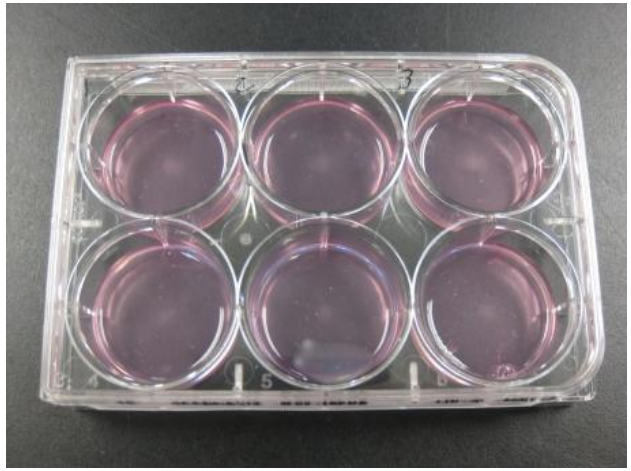


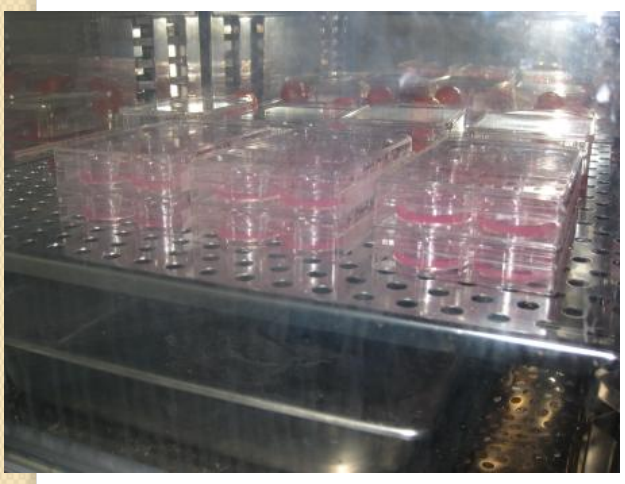
MNV-1 (QCT-2) (con't)











Plaques

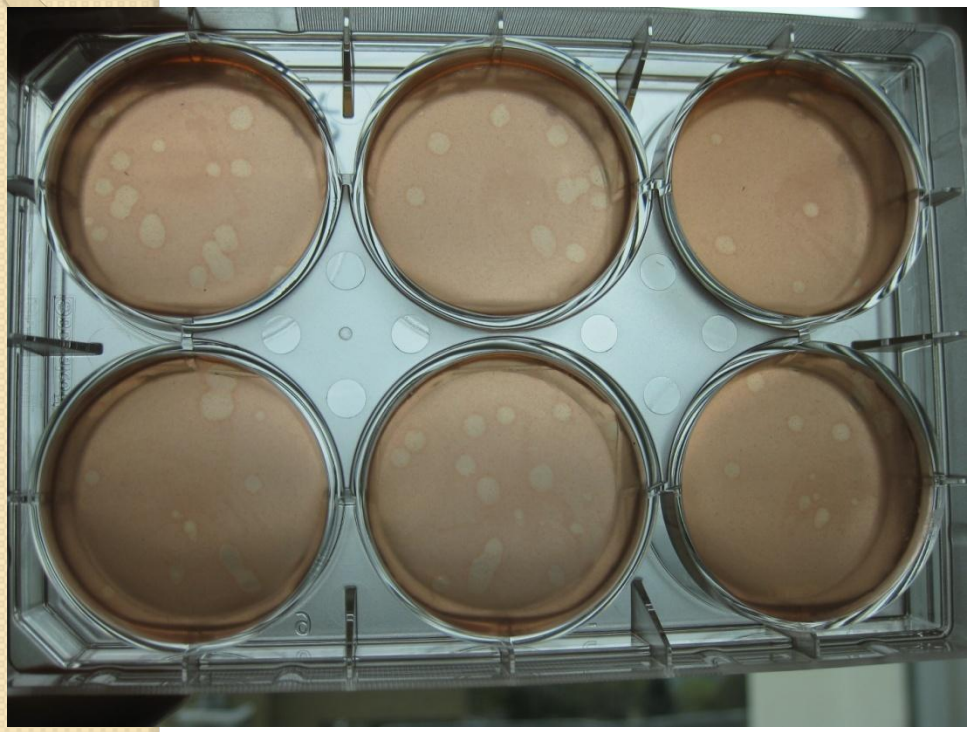


Figure 1. Sodium hypochlorite at 1 minute

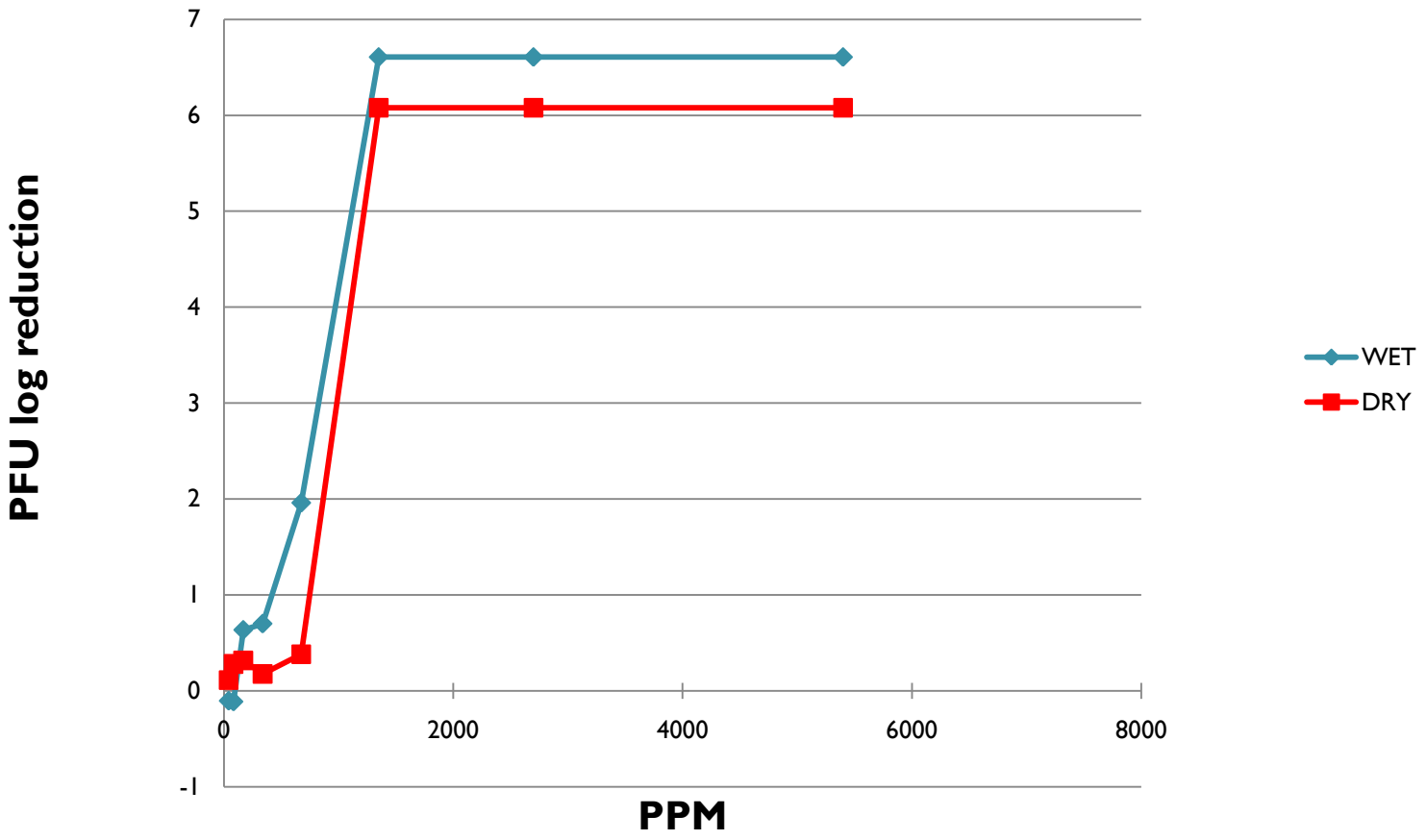


Figure 2. Sodium hypochlorite at 5 minutes

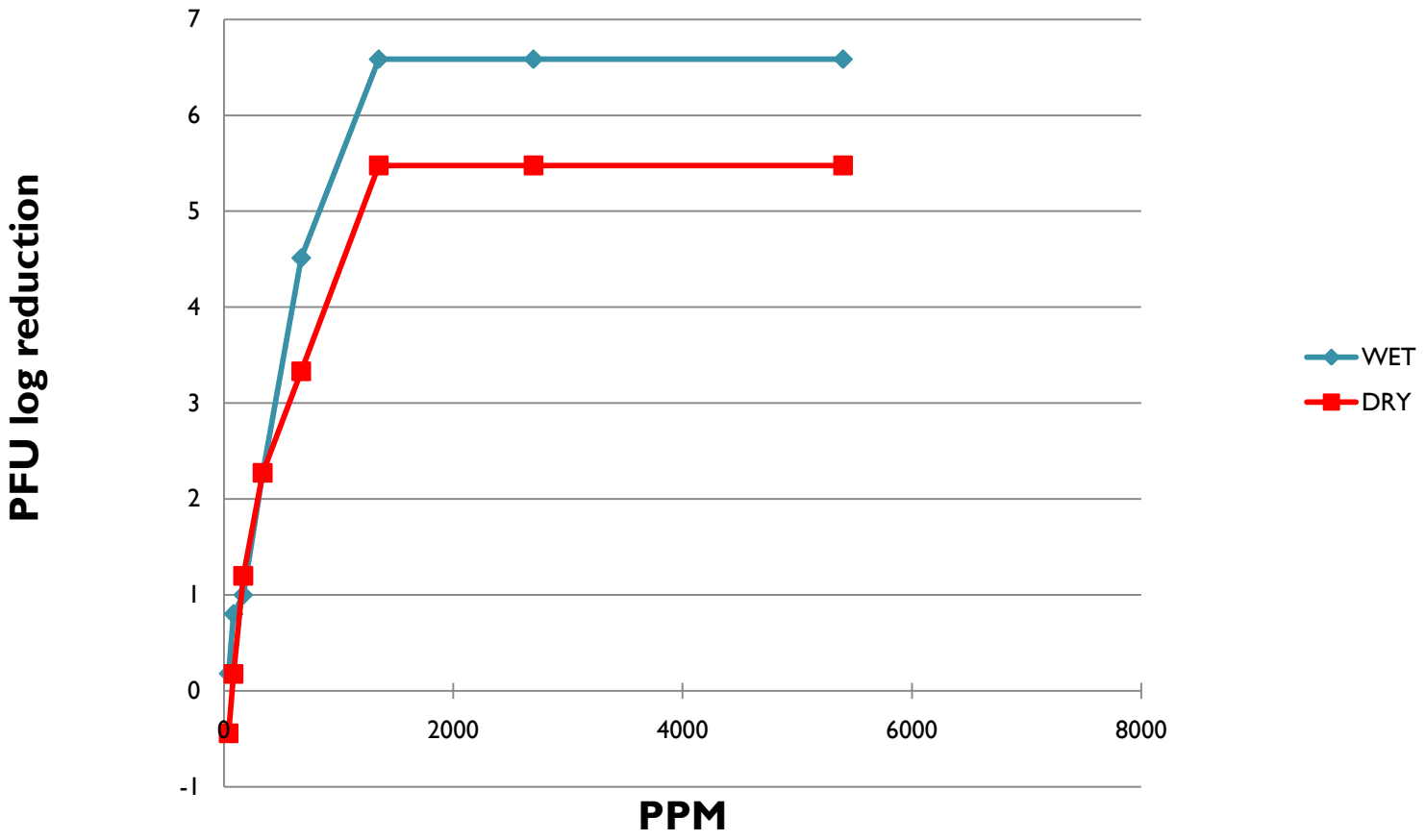
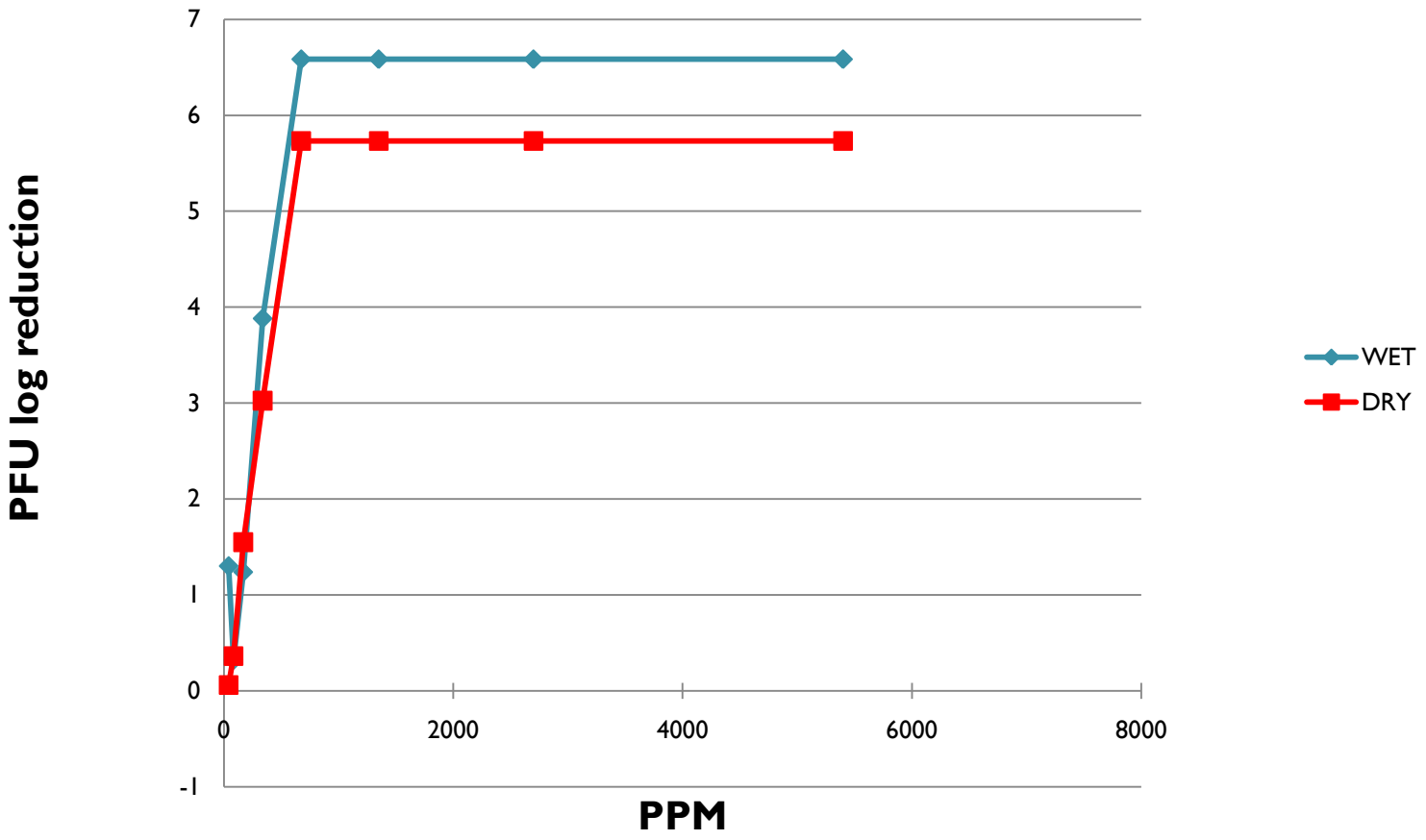


Figure 3. Sodium hypochlorite at 10 minutes

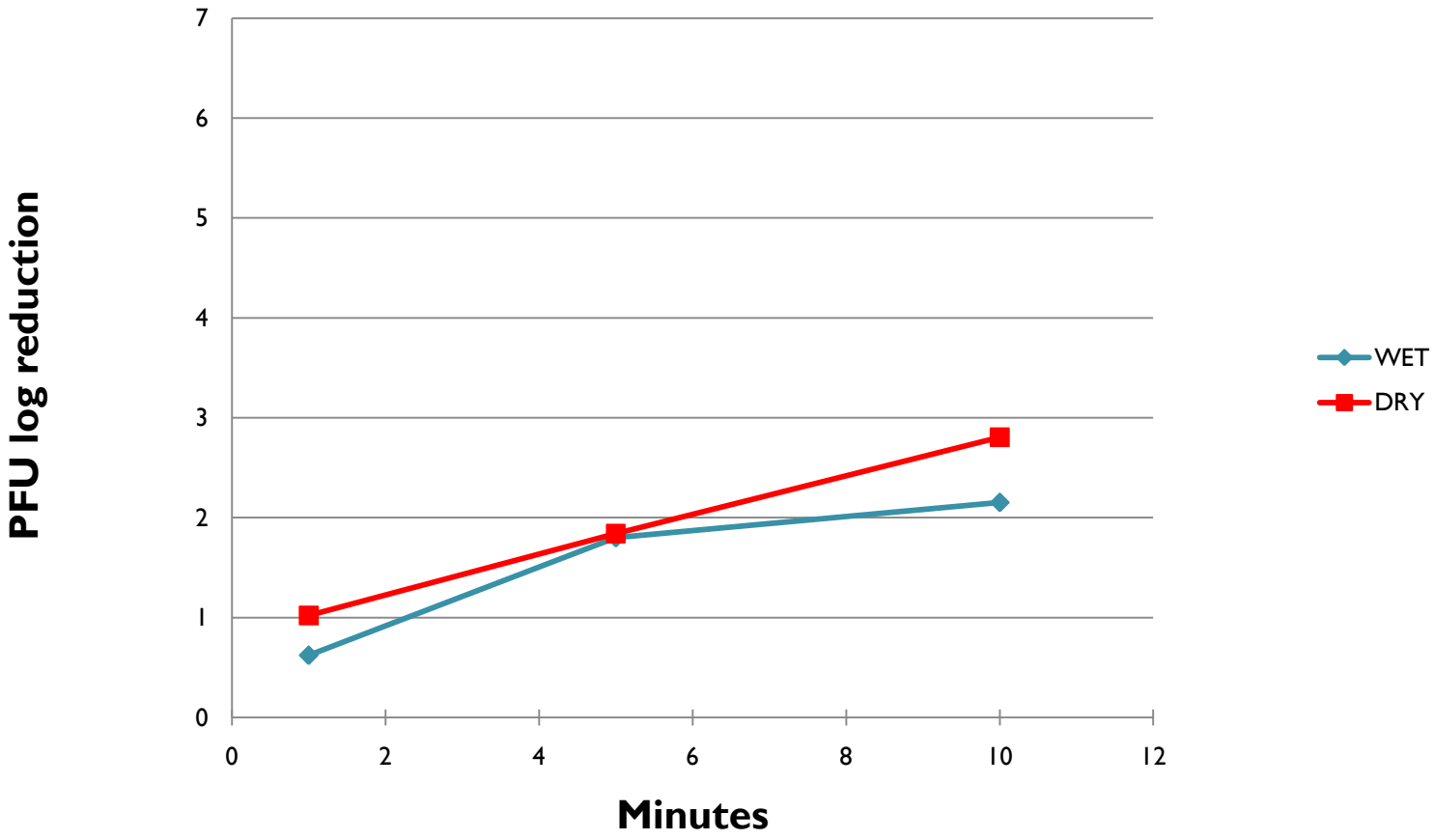


Sodium Hypochlorite Log Reduction Results

Dilution	PPM	Wet – log reduction			Dry – log reduction		
		1 min	5 min	10 min	1 min	5 min	10 min
1/10	5400	6.61	6.58	6.58	6.08	5.48	5.73
1/20	2700	6.61	6.58	6.58	6.08	5.48	5.73
1/40	1350	6.61	6.58	6.58	6.08	5.48	5.73
1/80	675	1.96	4.51	6.58	0.38	3.33	5.73
1/160	338	0.70	2.27	3.88	0.18	2.27	3.02
1/320	169	0.63	1.00	1.24	0.32	1.20	1.55
1/640	84	-0.11	0.80	0.32	0.28	0.18	0.36
1/1280	42	-0.11	0.18	1.30	0.11	-0.44	0.06

- Control range (wet load): 3.84 to 4.04×10^6 pfu/mL
- Control range (dry load): 3.00×10^5 to 1.2×10^6 pfu/mL
- 1 log reduction = 90 % reduction in population (of virus)

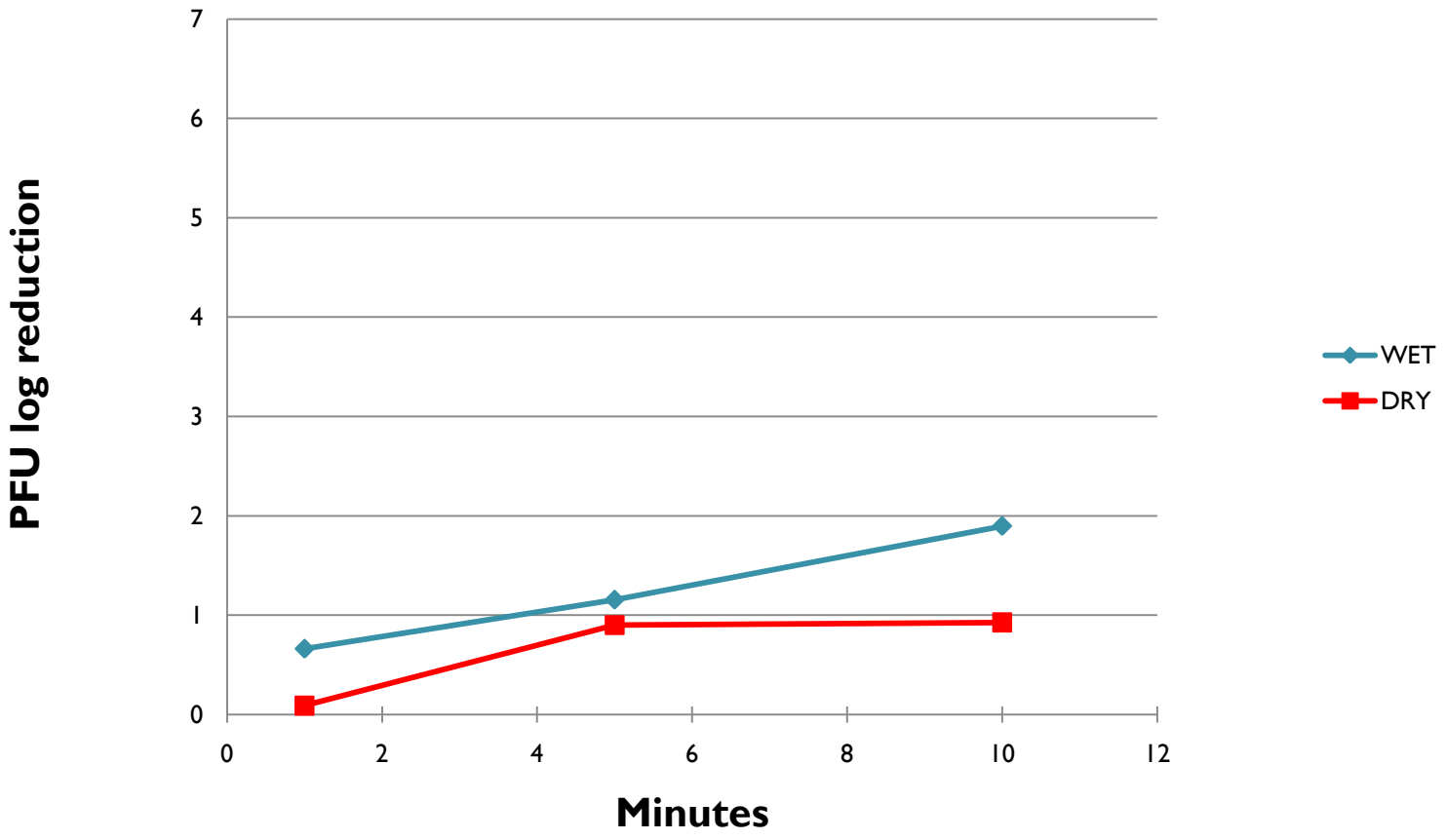
Figure 4. RTU Quaternary Ammonium at 1, 5, 10 minutes (2800 ppm)



Quaternary Ammonium Results (RTU strength 2800 ppm)

WET @ 1, 5 and 10 minutes					DRY @ 1, 5 and 10 minutes				
Time (min)	**PPM**	**PFU/mL**	**Control**	**Log Reduction**	**Time (min)**	**PPM**	**PFU/mL**	**Control**	**Log Reduction**
1	2800	7.47×10^5	3.13×10^6	0.62	1	2800	7.90×10^4	8.30×10^5	1.02
5	2800	5.45×10^4	3.43×10^6	1.80	5	2800	1.20×10^4	8.30×10^5	1.84
10	2800	2.42×10^4	3.43×10^6	2.15	10	2800	5.00×10^2	3.20×10^5	2.81

Figure 5. RTU Accelerated Hydrogen Peroxide at 1, 5 and 10 minutes (0.5 % hydrogen peroxide)



RTU Accelerated Hydrogen Peroxide Results (0.5 % hydrogen peroxide)

WET @ 1, 5 and 10 minutes					DRY @ 1, 5 and 10 minutes				
Time (min)	**PPM**	**PFU/mL**	**Control**	**Log Reduction**	**Time (min)**	**PPM**	**PFU/mL**	**Control**	**Log Reduction**
1	5000	2.42×10^5	1.11×10^6	0.66	1	5000	2.20×10^5	2.70×10^5	0.09
5	5000	7.78×10^4	1.11×10^6	1.15	5	5000	3.40×10^4	2.70×10^5	0.90
10	5000	1.41×10^4	1.11×10^6	1.90	10	5000	3.20×10^4	2.70×10^5	0.93

Figure 6. Accelerated Hydrogen Peroxide at 1 minute (7.0 % hydrogen peroxide)

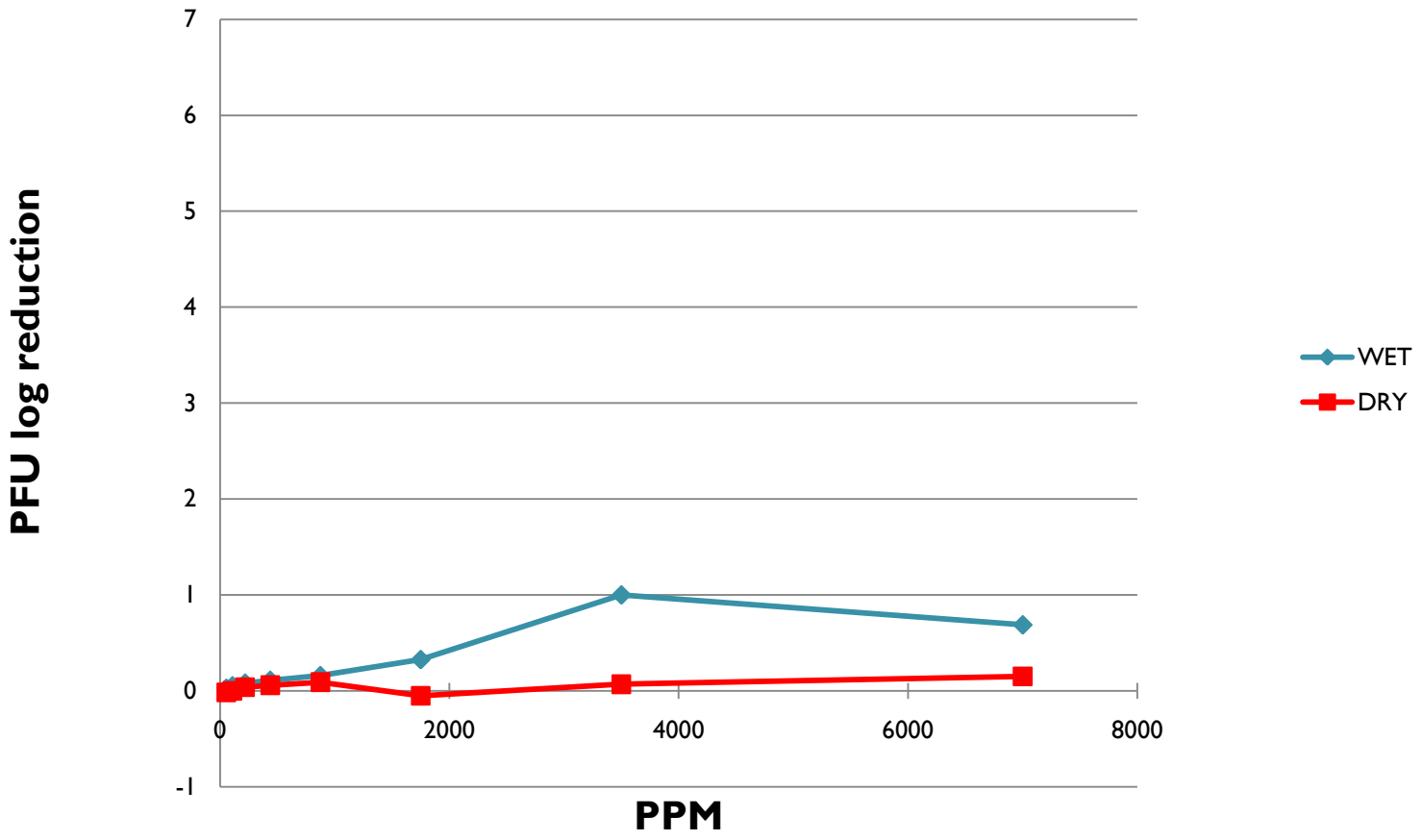


Figure 7. Accelerated Hydrogen Peroxide at 5 minutes (7.0 % hydrogen peroxide)

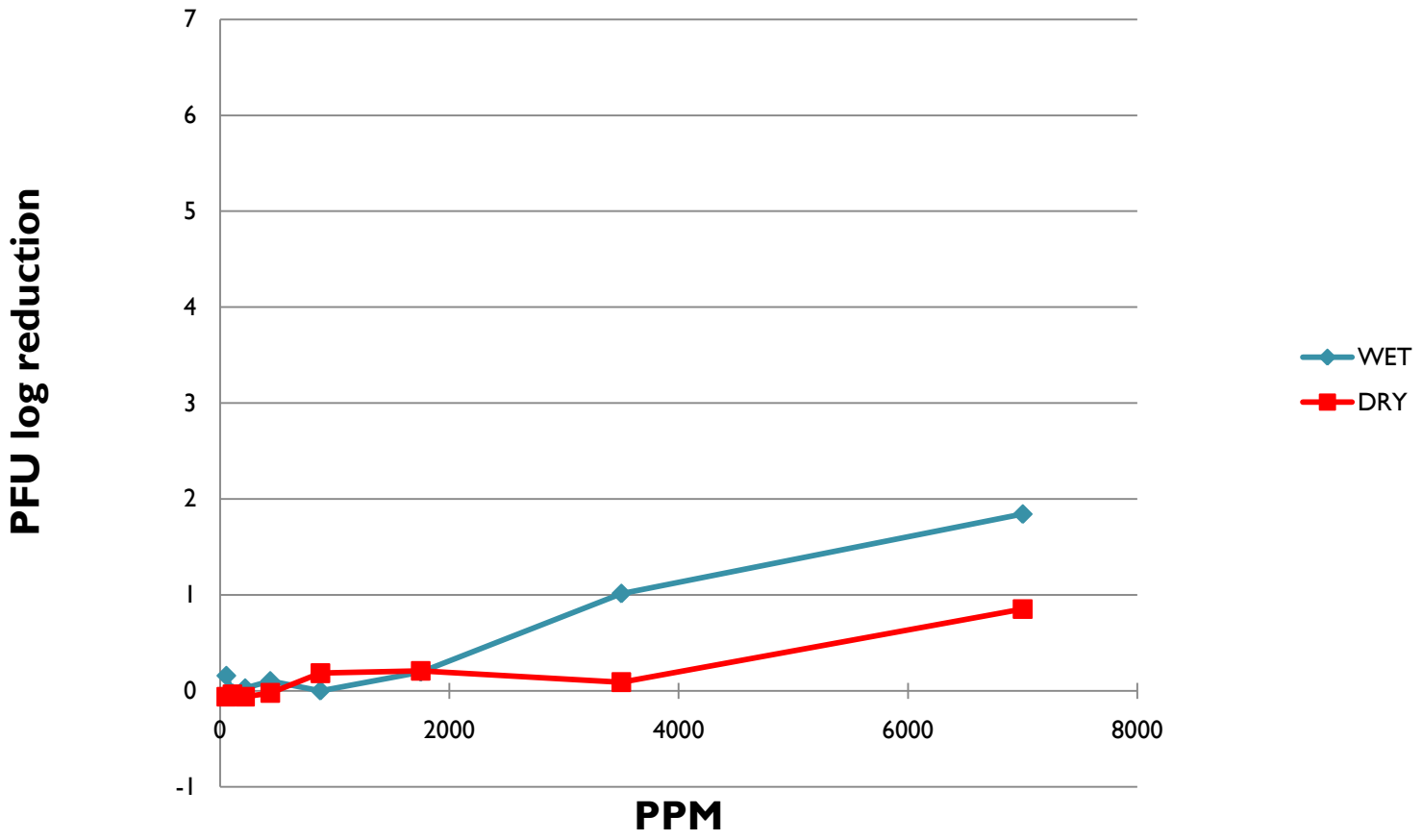
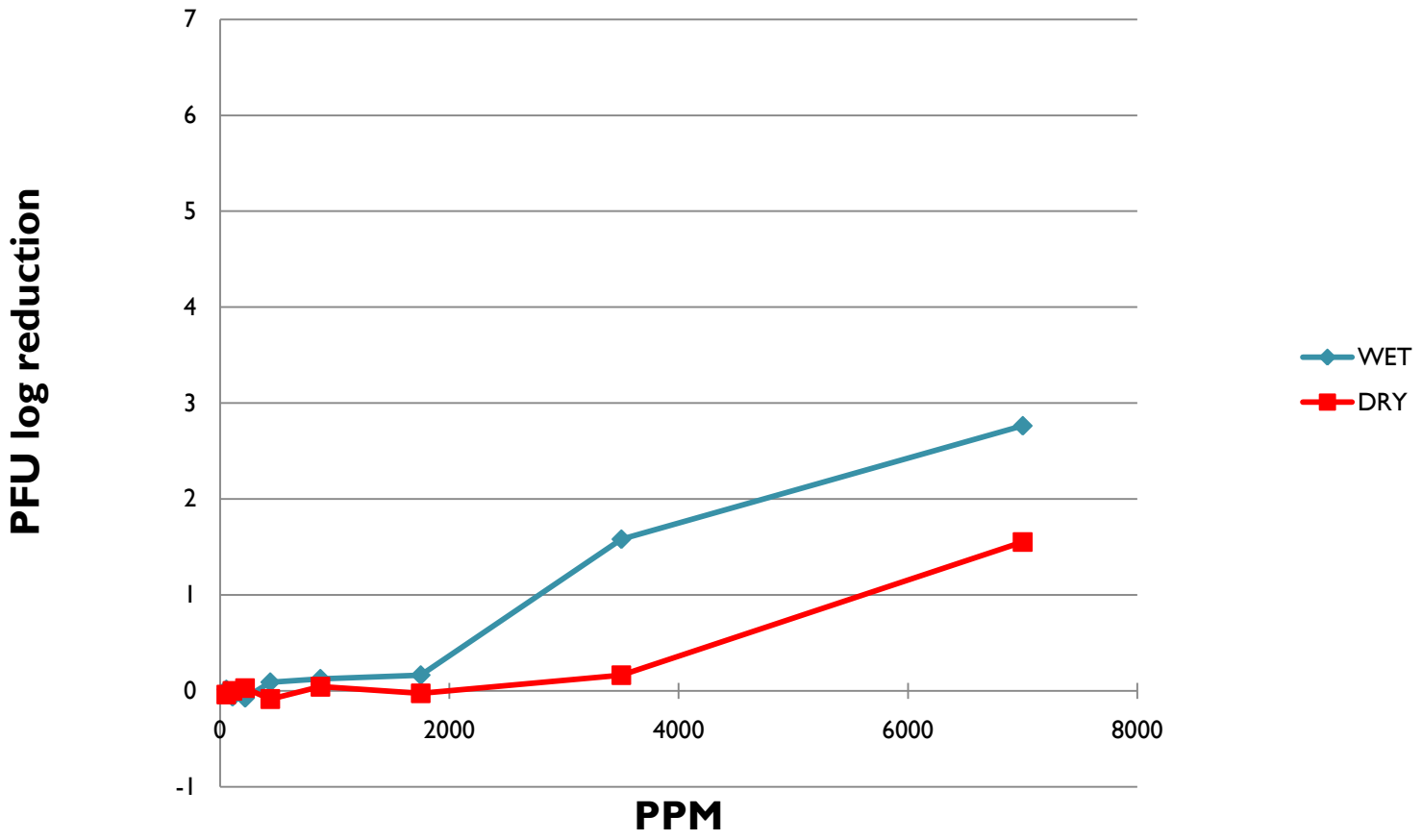


Figure 8. Accelerated Hydrogen Peroxide at 10 minutes (7.0 % hydrogen peroxide)



Summary of Results

- **Sodium hypochlorite** (1350 ppm or 1/40 dilution of 5.4 % bleach)
  - 1 minute: >6 log reduction
- **RTU quaternary ammonium cmpd** (2800 ppm or 0.28 %)
  - 5 minutes: <2 log reduction
  - 10 minutes: >2 log reduction
- **RTU accelerated hydrogen peroxide** (5000 ppm or 0.5 %)
  - 1 minute: <1 log reduction
  - 5 minutes: >1 log reduction
  - 10 minutes: <2 log reduction (wet), <1 log (dry)
- **Accelerated hydrogen peroxide** (7000 ppm or 0.7 %)
  - 1 minute: <1 log reduction
  - 5 minutes: <2 log reduction (wet), <1 log (dry)
  - 10 minutes: <3 log reduction (wet), <2 log (dry)

Summary

- Results demonstrate the interaction between concentration and time to have an effect on the efficacy of the disinfectant
- Work to date confirms the efficacy of sodium hypochlorite as a virucidal agent of MNV-1
- Work to date does not confirm the efficacy of quaternary ammonium and accelerated hydrogen peroxides as widely used in BC health care facilities

Future Work

- To test the disinfectants and cleaning agents against FCV as another surrogate for human norovirus
- Compare the efficacy of selected disinfectants with FCV and MNV-1, with and without soil load
- Experiments are a work in progress

Questions?