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

September 6, 2010

Stephanie Chiu

Judith Isaac-Renton, Brent Skura, Martin Petric, Bonnie Henry, Lorraine McIntyre, Bruce Gamage
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


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


Transmission

- **Fecal-oral route**
  - 10-100 virion particles to become infected

- **Person-to-person spread**
  - Aerosol formation – projectile vomiting
  - Up to $3 \times 10^7$ virus particles distributed as an aerosol

- **Foodborne transmission**
  - Oysters, other food

- **Waterborne transmission**

- **Inanimate environments**
  - Surfaces (e.g. taps, handles, sinks)
  - Medical equipment

---

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


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


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

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


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

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

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 Suspension with Soil Load**
- Transfer 10 µL to stainless steel disk and allow to dry for 0 H (WET) or 60-90 min. (DRY)
- Transfer disks onto bottom of glass vials
- **Test Carriers**
  - Add 50 µL disinfectant
  - Hold for 1, 5 and 10 min.
  - Add 950 µL neutralizer solution
  - Vortex
- Transfer to 2 mL vial and make 10 fold dilutions
- Add 500 µL aliquots to each well and incubate in room temp. for 1 h
- Add first overlay to each well and incubate at 37 °C in CO₂ incubator for 36 h
- Add second overlay (with neutral red) and incubate in 37 °C in CO₂ incubator overnight
- Visualize plaques and count as infective units

**MNV-1 Suspension without Soil Load**
- Transfer 10 µL to stainless steel disk and allow to dry for 0 H (WET) or 60-90 min. (DRY)
- Transfer disks onto bottom of glass vials
- **Control Carriers**
  - Add 50 µL DDH₂O
  - Hold for 1, 5 and 10 min.
  - Add 950 µL neutralizer solution
  - Vortex
- Transfer to 2 mL vial and make 10 fold dilutions
- Add 500 µL aliquots to each well and incubate in room temp. for 1 h
- Add first overlay to each well and incubate at 37 °C in CO₂ incubator for 36 h
- Add second overlay (with neutral red) and incubate in 37 °C in CO₂ incubator overnight
- Visualize plaques and count as infective units
MNV-1 (QCT-2) (con’t)
Plaques
Figure 1. Sodium hypochlorite at 1 minute

PFU log reduction vs. PPM
Figure 2. Sodium hypochlorite at 5 minutes
Figure 3. Sodium hypochlorite at 10 minutes

PFU log reduction vs PPM
# Sodium Hypochlorite Log Reduction Results

<table>
<thead>
<tr>
<th>Dilution</th>
<th>PPM</th>
<th>Wet – log reduction</th>
<th>Dry – log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 min</td>
<td>5 min</td>
</tr>
<tr>
<td>1/10</td>
<td>5400</td>
<td>6.61</td>
<td>6.58</td>
</tr>
<tr>
<td>1/20</td>
<td>2700</td>
<td>6.61</td>
<td>6.58</td>
</tr>
<tr>
<td>1/40</td>
<td>1350</td>
<td>6.61</td>
<td>6.58</td>
</tr>
<tr>
<td>1/80</td>
<td>675</td>
<td>1.96</td>
<td>4.51</td>
</tr>
<tr>
<td>1/160</td>
<td>338</td>
<td>0.70</td>
<td>2.27</td>
</tr>
<tr>
<td>1/320</td>
<td>169</td>
<td>0.63</td>
<td>1.00</td>
</tr>
<tr>
<td>1/640</td>
<td>84</td>
<td>-0.11</td>
<td>0.80</td>
</tr>
<tr>
<td>1/1280</td>
<td>42</td>
<td>-0.11</td>
<td>0.18</td>
</tr>
</tbody>
</table>

- 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)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>PPM</th>
<th>PFU/mL</th>
<th>Control</th>
<th>Log Reduction</th>
<th>Time (min)</th>
<th>PPM</th>
<th>PFU/mL</th>
<th>Control</th>
<th>Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2800</td>
<td>$7.47 \times 10^5$</td>
<td>$3.13 \times 10^6$</td>
<td>0.62</td>
<td>1</td>
<td>2800</td>
<td>$7.90 \times 10^4$</td>
<td>$8.30 \times 10^5$</td>
<td>1.02</td>
</tr>
<tr>
<td>5</td>
<td>2800</td>
<td>$5.45 \times 10^4$</td>
<td>$3.43 \times 10^6$</td>
<td>1.80</td>
<td>5</td>
<td>2800</td>
<td>$1.20 \times 10^4$</td>
<td>$8.30 \times 10^5$</td>
<td>1.84</td>
</tr>
<tr>
<td>10</td>
<td>2800</td>
<td>$2.42 \times 10^4$</td>
<td>$3.43 \times 10^6$</td>
<td>2.15</td>
<td>10</td>
<td>2800</td>
<td>$5.00 \times 10^2$</td>
<td>$3.20 \times 10^5$</td>
<td>2.81</td>
</tr>
</tbody>
</table>
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)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>PPM</th>
<th>PFU/mL</th>
<th>Control</th>
<th>Log Reduction</th>
<th>Time (min)</th>
<th>PPM</th>
<th>PFU/mL</th>
<th>Control</th>
<th>Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5000</td>
<td>$2.42 \times 10^5$</td>
<td>$1.11 \times 10^6$</td>
<td>0.66</td>
<td>1</td>
<td>5000</td>
<td>$2.20 \times 10^5$</td>
<td>$2.70 \times 10^5$</td>
<td>0.09</td>
</tr>
<tr>
<td>5</td>
<td>5000</td>
<td>$7.78 \times 10^4$</td>
<td>$1.11 \times 10^6$</td>
<td>1.15</td>
<td>5</td>
<td>5000</td>
<td>$3.40 \times 10^4$</td>
<td>$2.70 \times 10^5$</td>
<td>0.90</td>
</tr>
<tr>
<td>10</td>
<td>5000</td>
<td>$1.41 \times 10^4$</td>
<td>$1.11 \times 10^6$</td>
<td>1.90</td>
<td>10</td>
<td>5000</td>
<td>$3.20 \times 10^4$</td>
<td>$2.70 \times 10^5$</td>
<td>0.93</td>
</tr>
</tbody>
</table>
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-I.

• 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?