Evaluating Low Concentration Hydrogen Peroxide Vapor for Inactivation of Ebola Virus Surrogates Phi6 and MS2 Bacteriophage

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Acknowledgements and Disclaimer

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  – Leroy Mickelsen, Shannon Serre, Worth Calfee

• Performer: Battelle

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Electron micrograph 1976 Ebola virus isolate; Credit CDC/Dr. Frederick Murphy
Outline of Presentation

• Problem definition, purpose of study
• Overview of study
• Methods and results
• Summary

Demo of diagnostic test for Ebola virus; Guinea 2016; Credit CDC/A.K. Knipes
Problem Definition

• Effective decontamination techniques against the Ebola virus (EBOV) virus are needed because:
  – Current disease outbreaks
  – Ability of EBOV to persist in the environment under certain conditions

• Simple, easy-to-use, decontamination techniques such as low concentration hydrogen peroxide vapor (LCHP) may help in locations where specialized equipment and financial resources may be limited
Rationale for the Research

- Evaluate hydrogen peroxide vapor (HPV) effectiveness in inactivating two EBOV surrogates as a function of:
  - Low and high concentrations HPV
  - Presence of human blood
  - Material
  - Contact time
Microbiology

• EBOV is an enveloped virus, BSL4 bioagent
• Phi6 bacteriophage
  – Recommended as surrogate for EBOV in several studies
  – Lipid-enveloped virus like EBOV
  – Use of plaque assay with *Pseudomonas syringae* as host cells
Microbiology

- MS2 bacteriophage
  - Non-enveloped virus
  - Use *Escherichia coli* C-3000 as host cells
  - CDC recommends disinfectants used for EBOV be EPA-registered for non-enveloped viruses
  - Non-enveloped viruses are more difficult to inactivate than enveloped ones like EBOV
Microbiological methods

• Titer of 5 E7 PFU/mL
• Inoculate 5E6 PFU per coupon via 0.1 mL
• Dilution plating, plaque counting on agar in triplicate
• Viruses extracted with 10 mL sterile phosphate buffered saline (PBS); samples agitated 15 minutes at 200 rpm
• Incubate plates $26 \pm 2 ^\circ C$ (Phi6) or $37 \pm 2 ^\circ C$ (MS2) for 18-24 h
Hydrogen Peroxide Vapor

• Used low (25 ppm) and high concentrations (> 400 ppm) for testing

• Low concentration hydrogen peroxide vapor can be generated through off the shelf humidifiers and aqueous hydrogen peroxide solutions
  – Previous work has shown LCHP to be effective against *B. anthracis*, provided sufficient contact time
Test Materials

- Glass, Stainless Steel, Ceramic Tile, N95 Respirator Filter Media, Painted Joint Tape, Wood
Decontamination Efficacy

- Phages were recovered from positive controls (not exposed to HPV) at the same elapsed times as the decontaminated coupons to assess efficacy.
- Efficacy calculated as log reduction.
- For virucidal claims, US EPA requires disinfectants demonstrate > 3 LR.
Persistence of the Viruses

- Positive control data were also be used to provide an indication of the environmental stability (persistence) of the phages
- Positive controls stored at ambient conditions ~ 22 °C

CDC Sally Ezra 2014 Nigeria
# Study Test Matrix Overview

<table>
<thead>
<tr>
<th>Virus</th>
<th>Test Materials</th>
<th>Diluent</th>
<th>Target Decontamination Conditions</th>
<th>Time points assessed (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phi6</td>
<td>Glass, Stainless Steel, Ceramic Tile, N95 Media, Painted Joint Tape, Wood</td>
<td>Blood</td>
<td>25 ppm, 75% RH</td>
<td>2,4,24,72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PBS</td>
<td>25 ppm, 75% RH</td>
<td>2,4,6,8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>400 ppm, 75% RH</td>
<td>4,8,24,32</td>
</tr>
<tr>
<td>MS2</td>
<td>Glass, Stainless Steel, Ceramic Tile, N95 Media, Painted Joint Tape, Wood</td>
<td>Blood</td>
<td>25 ppm, 75% RH</td>
<td>2,4,8,24,32,72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PBS</td>
<td>25 ppm, 75% RH</td>
<td>2,4,6,8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>400 ppm, 75% RH</td>
<td>4,8,24,32</td>
</tr>
</tbody>
</table>
Results - Persistence of Phi6 in PBS
Results - Persistence of Phi6 in blood

![Bar graph showing the persistence of Phi6 in blood over time for different materials: glass, SS, tile, N95, PJT, and wood. The graph plots log PFU recovered against hours (0, 24, 72) on a logarithmic scale.]
Results - Persistence of MS2 in PBS
Results - Persistence of MS2 in blood

Log PFU recovered vs Hours:
- 0 hours inoculum
- 2 hours
- 72 hours

Materials:
- glass
- SS
- tile
- N95
- PJT
- wood
## Decon Results for Phi 6

<table>
<thead>
<tr>
<th>Diluent</th>
<th>HPV ppm</th>
<th>Time Point (h)</th>
<th>Glass</th>
<th>SS</th>
<th>Tile</th>
<th>N95</th>
<th>PJT</th>
<th>Wood</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>25</td>
<td>2</td>
<td>4.3 ± 1.9</td>
<td>4.2 ± 1.6</td>
<td>&gt;5.7 ± 0.2</td>
<td>&gt;1.6 ± 1.6</td>
<td>&gt;0.0</td>
<td>-0.6 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>4.3 ± 1.5</td>
<td>5.1 ± 1.0</td>
<td>&gt;3.6 ± 2.1</td>
<td>&gt;1.3 ± 1.4</td>
<td>&gt;0.00</td>
<td>&gt;0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>&gt;5.9 ± 0.1</td>
<td>&gt;5.6 ± 0.1</td>
<td>&gt;6.1 ± 0.1</td>
<td>&gt;0.8 ± 1.6</td>
<td>&gt;0.0 ± 0.0</td>
<td>&gt;0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>&gt;5.6 ± 0.1</td>
<td>&gt;3.6 ± 1.1</td>
<td>&gt;5.6 ± 0.2</td>
<td>&gt;2.4 ± 0.4</td>
<td>&gt;0.8 ± 1.6</td>
<td>&gt;0.0 ± 0.0</td>
</tr>
<tr>
<td>Blood</td>
<td>25</td>
<td>24</td>
<td>-0.1 ± 0.2</td>
<td>-0.1 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>-0.2 ± 0.2</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Blood</td>
<td>429</td>
<td>4</td>
<td>0.3 ± 0.1</td>
<td>-0.2 ± 0.4</td>
<td>0.1 ± 0.2</td>
<td>0.7 ± 0.4</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>-0.1 ± 0.2</td>
<td>-0.0 ± 0.1</td>
<td>-0.4 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>5.2 ± 1.3</td>
<td>4.1 ± 0.4</td>
<td>5.9 ± 1.3</td>
<td>5.2 ± 1.3</td>
<td>6.7 ± 0.1</td>
<td>3.7 ± 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>5.5 ± 1.0</td>
<td>6.7 ± 0.1</td>
<td>4.8 ± 0.3</td>
<td>5.6 ± 1.0</td>
<td>5.9 ± 1.2</td>
<td>6.4 ± 0.1</td>
</tr>
</tbody>
</table>
# Decon Results for MS2

<table>
<thead>
<tr>
<th>Diluent</th>
<th>HPV ppm</th>
<th>Time Point (h)</th>
<th>Average Decontamination Efficacy (Log Reduction ± 95% CI limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glass</td>
</tr>
<tr>
<td>PBS</td>
<td>25</td>
<td>2</td>
<td>3.6 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>3.5 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>&gt;5.2 ± 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>&gt;4.4 ± 0.7</td>
</tr>
<tr>
<td>Blood</td>
<td>25</td>
<td>24</td>
<td>0.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72</td>
<td>0.7 ± 1.2</td>
</tr>
<tr>
<td>Blood</td>
<td>454</td>
<td>4</td>
<td>1.1 ± 1.1</td>
</tr>
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<td></td>
<td></td>
<td>8</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>&gt;2.9 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>&gt;3.2 ± 0.3</td>
</tr>
</tbody>
</table>
Summary for Phage Persistence

• Both phages recovered from all positive control materials at longest time point of 8 h in PBS
  – Except Phi6 from PJT and wood

• Both phages recovered from all positive control materials at 72 h (longest time point in the presence of human blood)

• In human blood, the persistence of the Phi6 enveloped phage was prolonged and masked the effect of material
  – This effect of blood was not as evident in the recovery of the non-enveloped MS2 phage
Summary for Decon Results

• LCHP was effective against both EBOV surrogates on all materials without the presence of blood at 2 h, for the phages that persisted that long

• LCHP was ineffective against the phages in the presence of blood, on all materials, even with a 3-day contact time

• Higher concentrations of HPV (> 400 ppmv) with contact times of 24-32 h achieved approximately 2-6 log reduction of the phages in the presence of blood