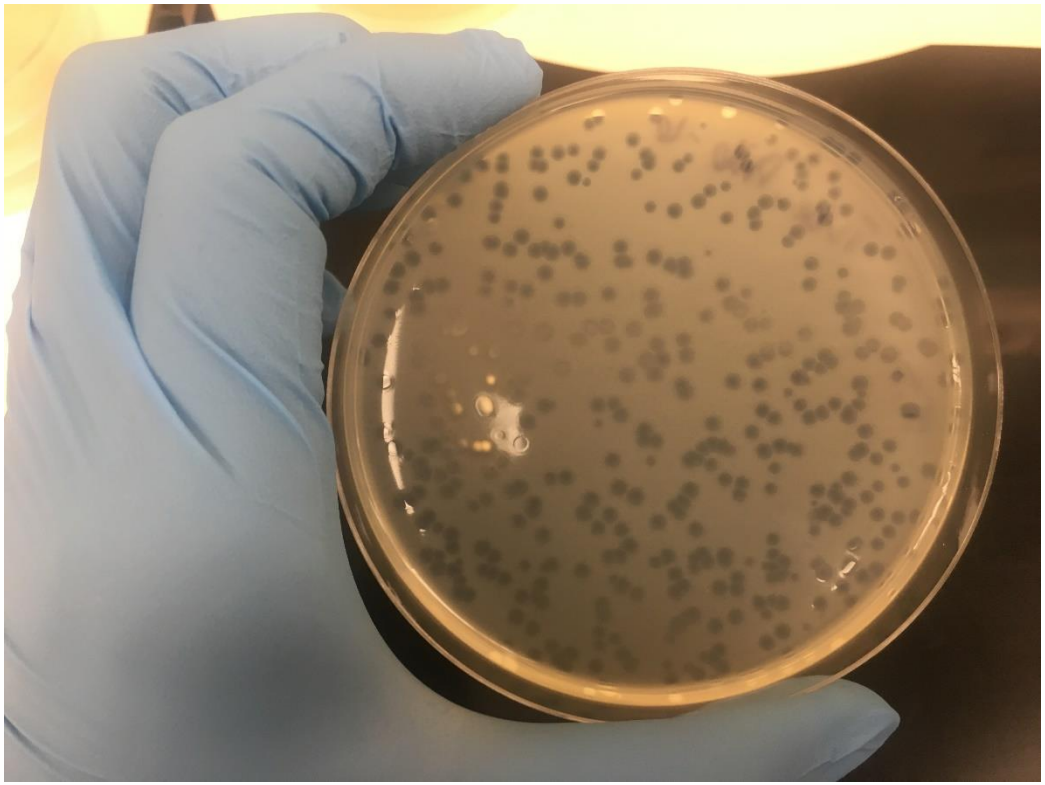
Evaluating Low Concentration Hydrogen Peroxide Vapor for Inactivation of Ebola Virus Surrogates Phi6 and MS2 Bacteriophage



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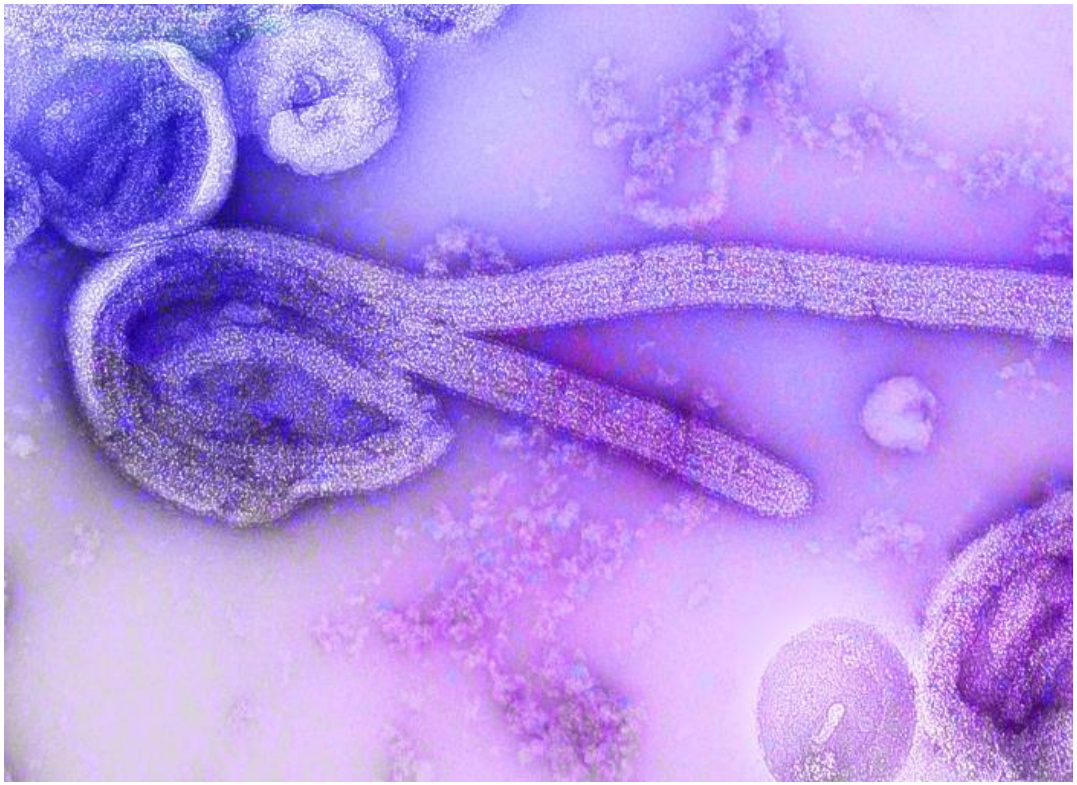
Presented at the US EPA Decontamination Conference, Norfolk, VA, November 19-21, 2019

Acknowledgements and Disclaimer

- EPA advisory team:
  - Leroy Mickelsen, Shannon Serre, Worth Calfee
- Performer: Battelle



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

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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

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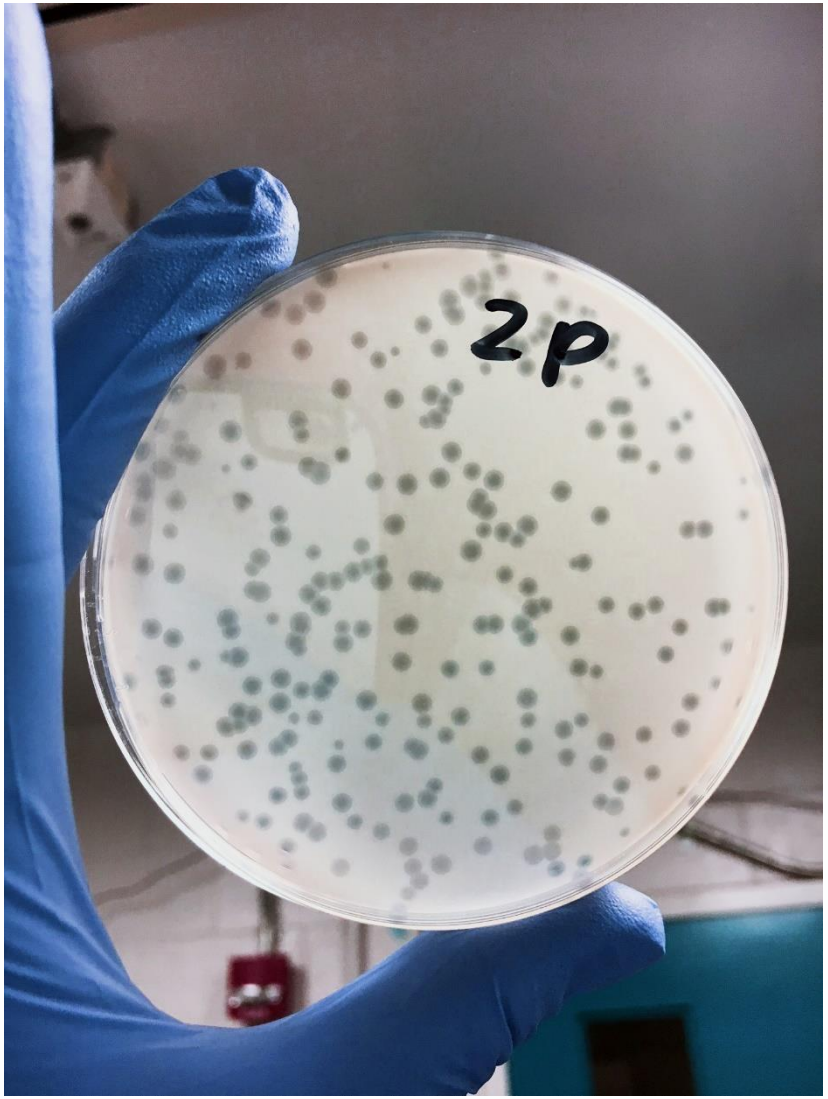
Rationale for the Research

- 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

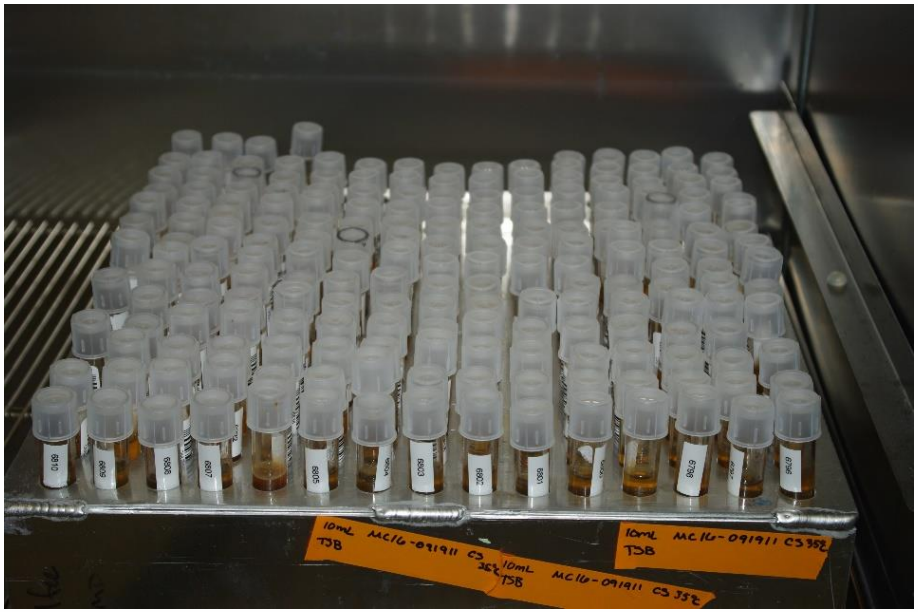
Microbiology

- 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



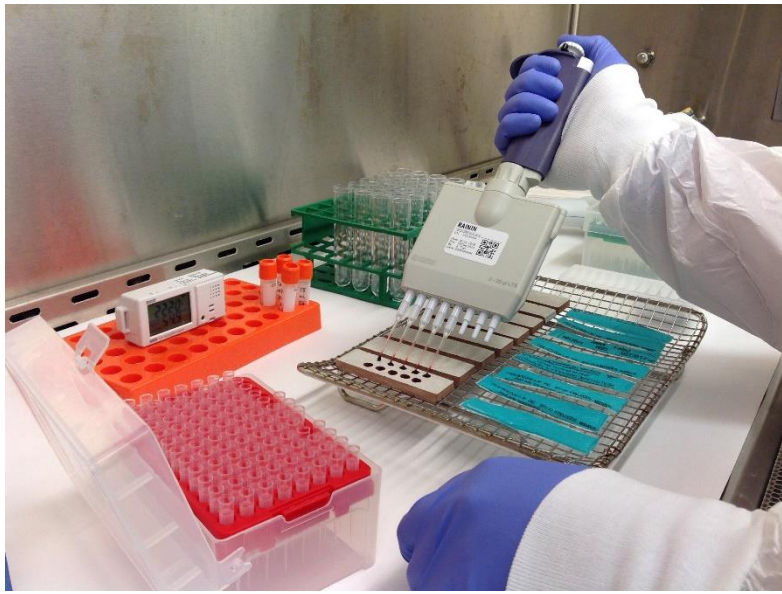
Microbiology

- 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 ± 2 ° C (Phi6) or 37 ± 2 ° C (MS2) for 18-24 h

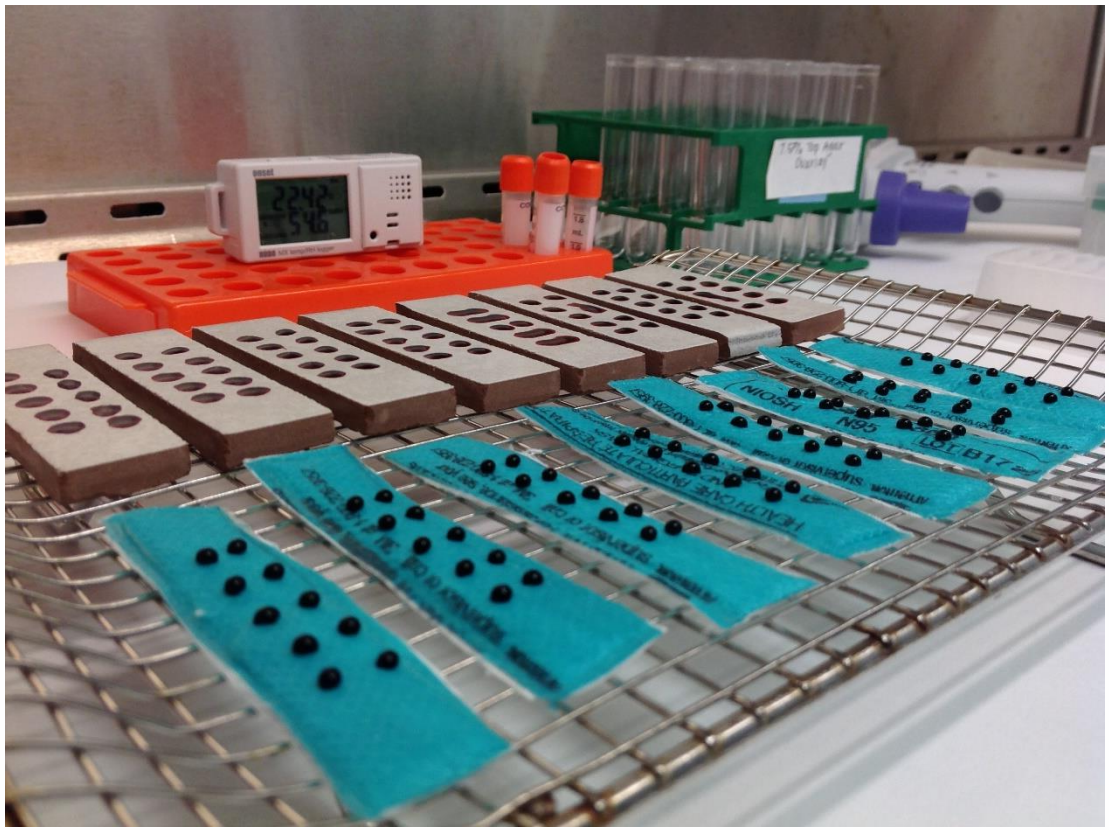


Hydrogen Peroxide Vapor

- 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

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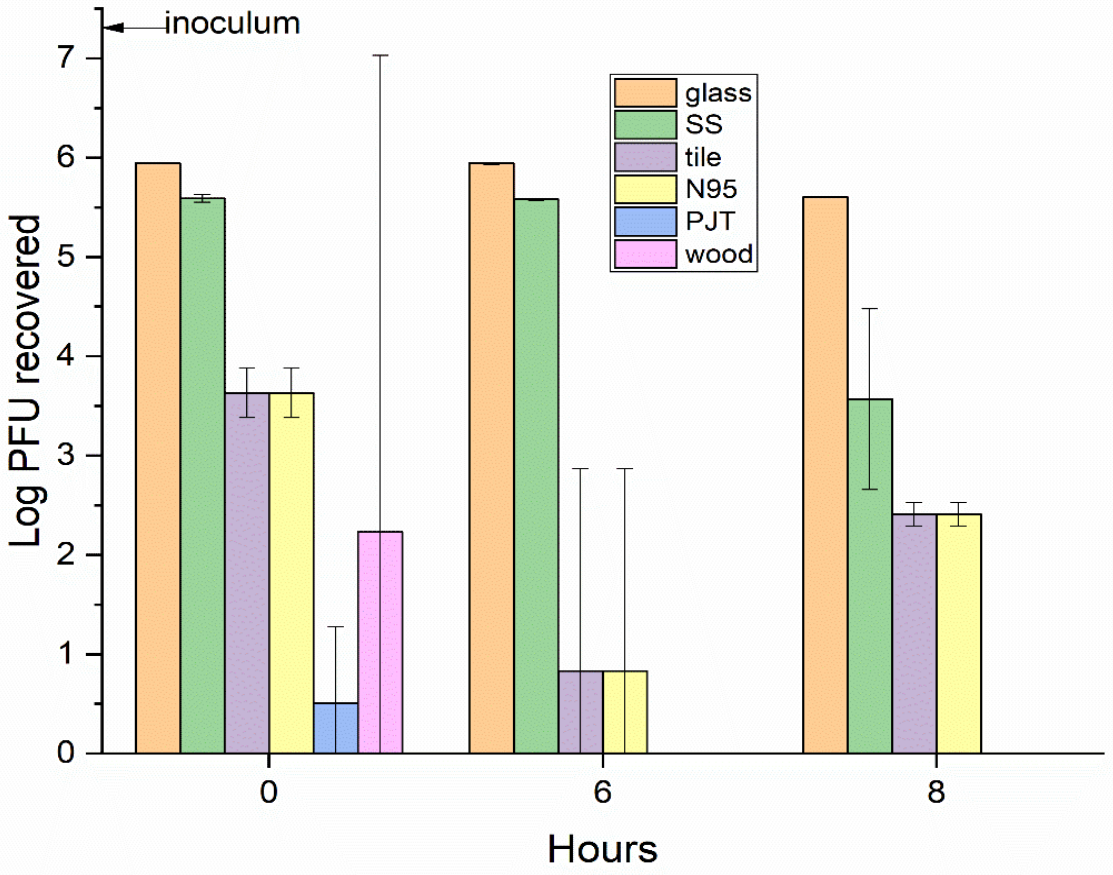
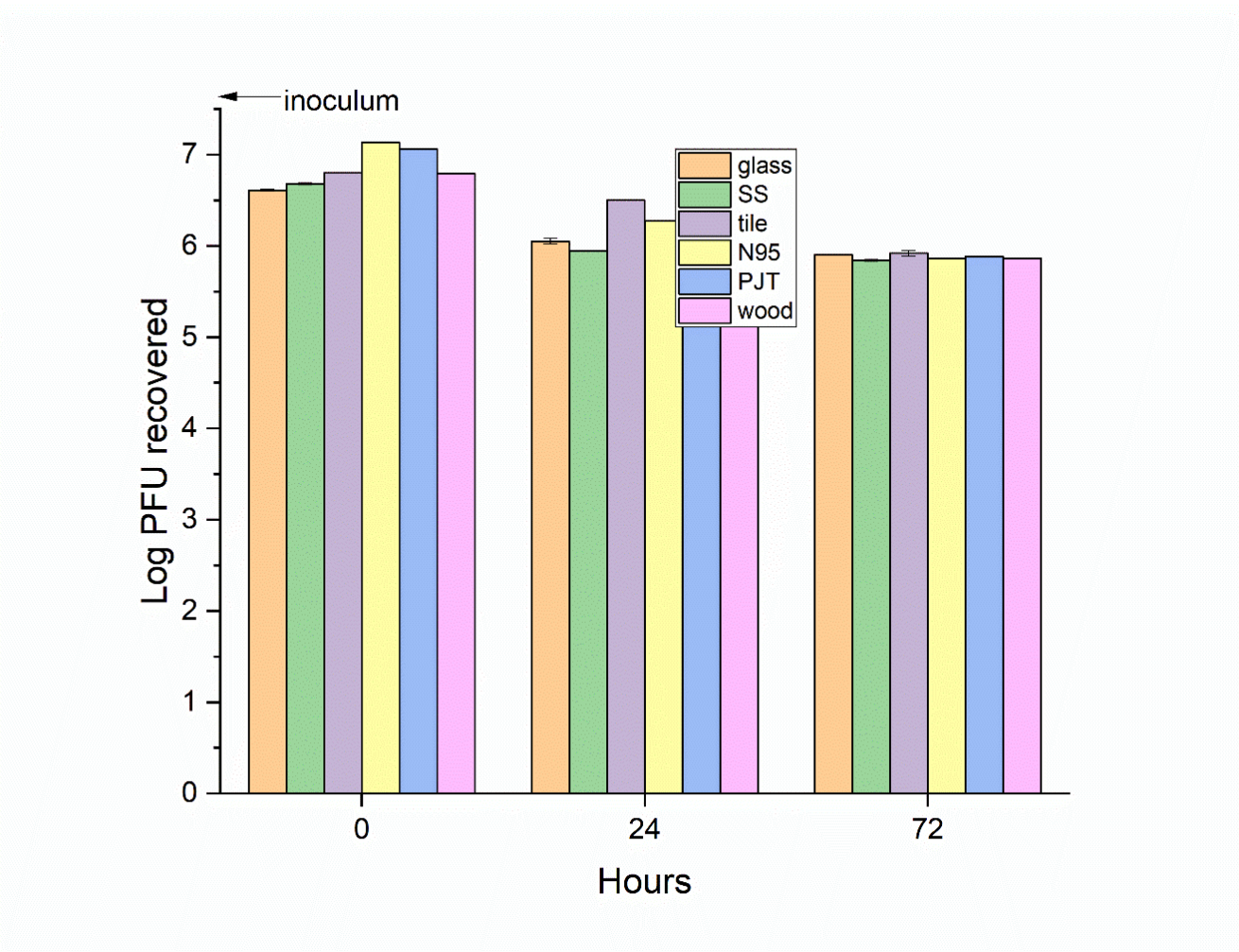
Study Test Matrix Overview

Virus	Test Materials	Diluent	Target Decontamination Conditions	Time points assessed (h)
Phi6	Glass, Stainless Steel, Ceramic Tile, N95 Media, Painted Joint Tape, Wood	Blood	25 ppm, 75% RH	2,4,24,72
		PBS	25 ppm, 75% RH	2,4,6,8
		Blood	400 ppm, 75% RH	4,8,24,32
MS2	Glass, Stainless Steel, Ceramic Tile, N95 Media, Painted Joint Tape, Wood	Blood	25 ppm, 75% RH	2,4,8,24,32,72
		PBS	25 ppm, 75% RH	2,4,6,8
		Blood	400 ppm, 75% RH	4,8,24,32

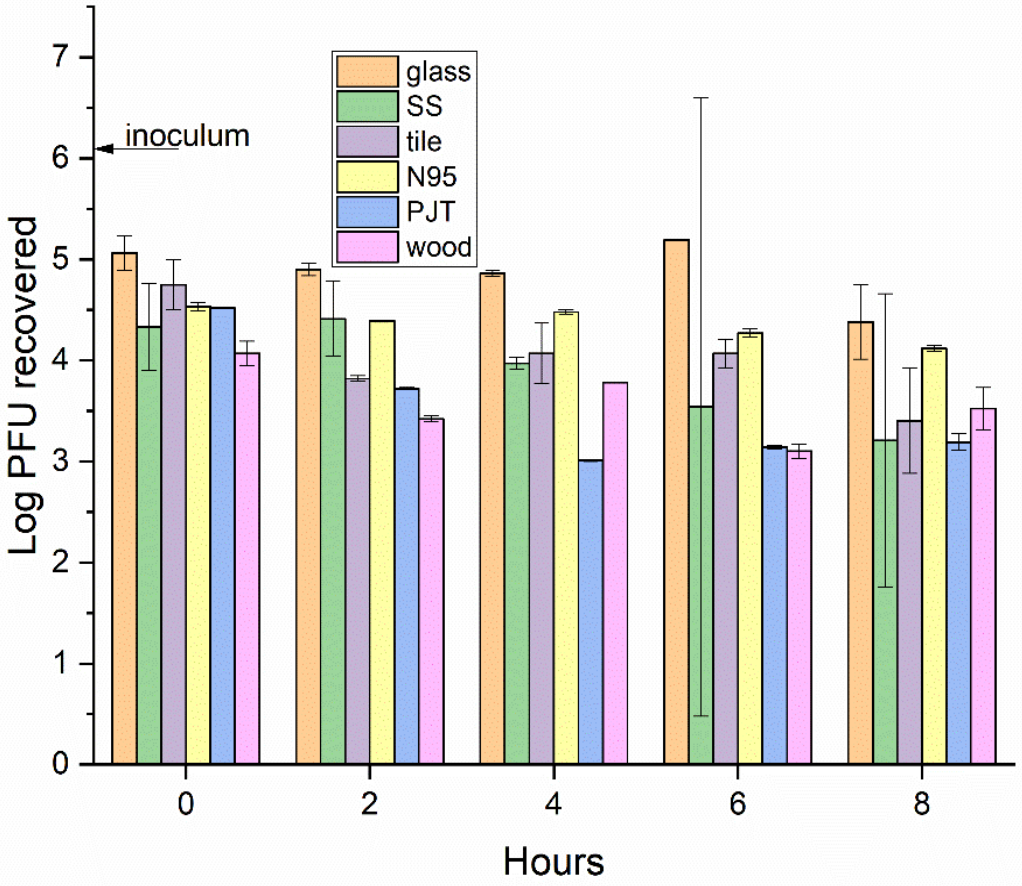
Results - Persistence of Phi6 in PBS



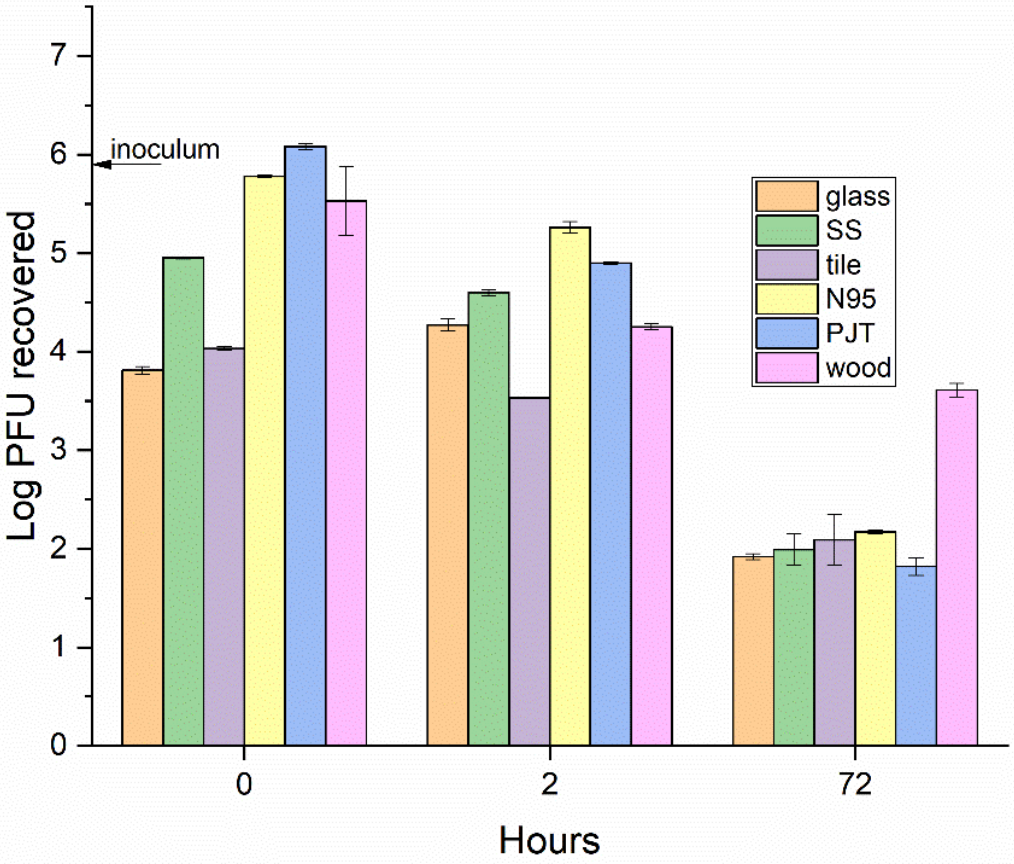
Results - Persistence of Phi6 in blood



Results - Persistence of MS2 in PBS



Results - Persistence of MS2 in blood



Decon Results for Phi 6

Diluent	HPV ppm	Time Point (h)	Average Decontamination Efficacy (Log Reduction ± 95% CI limits)					
			Glass	SS	Tile	N95	PJT	Wood
PBS	25	2	4.3 ± 1.9	4.2 ± 1.6	>5.7 ± 0.2	>1.6 ± 1.6	>0.0	-0.6 ± 1.2
		4	4.3 ± 1.5	5.1 ± 1.0	>3.6 ± 2.1	>1.3 ± 1.4	>0.00	>0.00
		6	>5.9 ± 0.1	>5.6 ± 0.1	>6.1 ± 0.1	>0.8 ± 1.6	> 0.0 ± 0.0	> 0.0 ± 0.0
		8	>5.6 ± 0.1	>3.6 ± 1.1	>5.6 ± 0.2	>2.4 ± 0.4	> 0.8 ± 1.6	> 0.0 ± 0.0
Blood	25	24	-0.1 ± 0.2	-0.1 ± 0.1	0.6 ± 0.2	0.1 ± 0.1	-0.2 ± 0.2	0.4 ± 0.4
		72	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
Blood	429	4	0.3 ± 0.1	-0.2 ± 0.4	0.1 ±0.2	0.7 ± 0.4	0.8 ± 0.3	0.8 ± 0.2
		8	-0.1 ± 0.2	- 0.0 ± 0.1	-0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.1	0.7 ± 0.1
		24	5.2 ± 1.3	4.1 ± 0.4	5.9 ± 1.3	5.2 ± 1.3	6.7 ± 0.1	3.7 ± 3.0
		32	5.5 ± 1.0	6.7 ± 0.1	4.8 ± 0.3	5.6 ± 1.0	5.9 ± 1.2	6.4 ± 0.1

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Decon Results for MS2

Diluent	HPV ppm	Time Point (h)	Average Decontamination Efficacy (Log Reduction ± 95% CI limits)					
			Glass	SS	Tile	N95	PJT	Wood
PBS	25	2	3.6 ± 1.3	>4.4 ± 0.7	>3.8 ± 0.2	>4.4 ± 0.1	>3.7 ± 0.1	>3.4 ± 0.2
		4	3.5 ± 1.3	>4.0 ± 0.3	>3.6 ± 1.2	>4.5 ± 0.2	>3.0 ± 0.1	>3.8 ± 0.1
		6	>5.2 ± 0.0	>3.5 ± 2.0	>4.1 ± 0.4	>4.3 ± 0.2	>3.1 ± 0.2	>3.1 ± 0.3
		8	>4.4 ± 0.7	>3.2 ± 1.4	>3.4 ± 0.8	>4.1 ± 0.2	>3.2 ± 0.3	>3.5 ± 0.5
Blood	25	24	0.2 ± 0.6	0.3 ± 0.3	0.4 ± 0.1	-0.1 ± 0.3	0.4 ± 0.3	0.5 ± 0.4
		32	0.4 ± 0.3	0.1 ± 0.5	0.4 ± 0.2	0.1 ± 0.3	0.8 ± 0.3	0.4 ± 0.2
		72	0.7 ± 1.2	1.2 ± 1.6	2.1 ± 0.6	0.9 ± 1.2	-0.3 ± 0.4	1.1 ± 0.6
Blood	454	4	1.1 ± 1.1	0.8 ± 0.5	0.2 ± 0.3	1.4 ± 1.6	0.6 ± 0.1	1.3 ± 0.2
		8	1.5 ± 1.5	1.2 ± 1.3	2.3 ± 1.2	1.6 ± 1.0	1.5 ± 0.3	1.2 ± 0.1
		24	>2.9 ± 0.7	>2.3 ± 0.3	>2.7 ± 0.3	>2.3 ± 0.2	>2.0 ± 1.4	3.3 ± 1.0
		32	>3.2 ± 0.3	>2.4 ± 0.3	>2.6 ± 0.7	>2.6 ± 0.4	2.9 ± 0.3	2.6 ± 1.2

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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

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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