Disinfectants against ASF virus: efficacy evaluation

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Plum Island Animal Disease Center Overview

Mission

Plum Island Animal Disease Center (PIADC) protects U.S. livestock from the accidental or intentional introduction of foreign animal diseases (FADs) that seriously threaten our livestock industries, food security and economy.

PIADC is the only federal, national laboratory that enhances current capabilities and evaluates state-of-the-art countermeasures (vaccines, diagnostics, disinfectants) through collaboration between DHS S&T, USDA ARS and USDA APHIS for high consequence transboundary animal diseases (TADs).

PIADC African Swine Fever Task Force

In response to ASF's increasing global threat PIADC established a DHS-USDA interagency ASF Task Force. The goal of the Task Force is to lead initiatives to:

- Fast-track development and scaled up production of an emergency use ASF vaccine;
- Improve diagnostic test surge capacity to support national surveillance and outbreak response;
- Evaluate commercially available disinfectants to determine their efficacy to decontaminate ASF virus contaminated spaces to support outbreak response capabilities and disinfection efforts.

DHS Applied Biosecurity Projects

- Provide actionable scientific data to support the "Response & Recovery" phase of TAD outbreaks
  - Virus elimination through cleaning and disinfection (C&D)
  - 3D (depopulation, disposal, and decontamination) projects

- Stakeholder Driven Testing & Evaluation (T&E)
  - Evaluation of commercial disinfectants used by swine producers (National Pork Board)
  - Evaluation of specialized disinfectants for use on sensitive aircraft surfaces (APHIS)
  - Method development: improvement of virus recovery from difficult surfaces (porous concrete) for chemical efficacy evaluation

ASF Virus Background: Virus Structure & Persistence

- ASFV: large, double-stranded DNA virus; genus *Asfivirus*, family *Asfarviridae*
  - Complex structure (2 capsids and 2 membranes)

- Resistance & Persistence
  - Viable for long periods in blood, tissues, and organic material including feed
  - Resistant to low temperatures (56ºC >1hr required for inactivation)
  - pH: requires acidic pH of <3.9 or basic pH of >11.5

  (OIE, 2019. ASF Technical Disease Card)

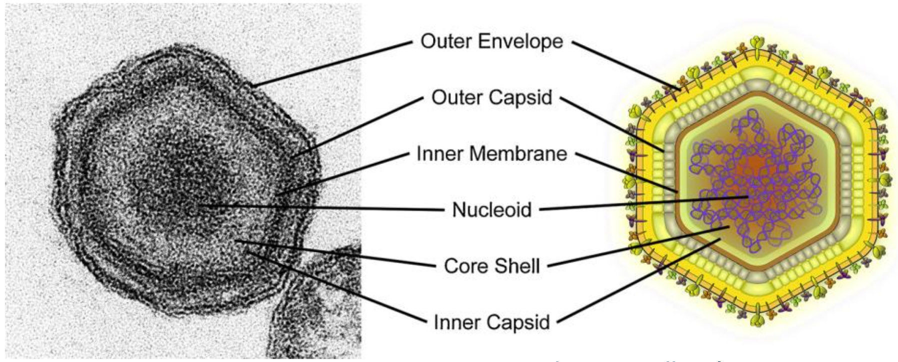


Photo Credit: Blome, S. 2020

Source: Virox Animal Health

ASF Virus Background: Biosecurity

- Primary control measures include enhanced biosecurity protocols to control virus spread geographically
  - Virus elimination through cleaning and disinfection
  - Decontamination of mechanical fomites (transport vehicles, clothing, feed)

Question: How do we know what chemicals are effective?

U.S. Registration of Chemical Disinfectants

- U.S. Environmental Protection Agency (EPA) regulates product registration under the Federal Insecticide & Rodenticide Act (FIFRA)
  - FIFRA Section 3
  - FIFRA Section 18

- All chemical disinfectants must demonstrate virucidal efficacy against ASFV

USDA APHIS Approved Disinfectants for use Against ASFV

Reg.Type	Product Name	EPA Reg.#	Manufacturer	Active Ingredient(s)	Contact Time
FIFRA Section 3	Virkon S	39967-137	Lanxess Co.	Sodium chloride Potassium peroxymonosulfate	10 min
FIFRA Section 3	Clearon Bleach Tablets	69470-37	Clearon Corp	Sodium dichloro-s-triazinetrione	30 min
FIFRA Section 3	Klor-Kleen	71847-2	Medentech Ltd.	Sodium dichloro-s-triazinetrione	30 min
FIFRA Section 3	Klorsept	71847-6	Medentech Ltd.	Sodium dichloro-s-triazinetrione	30 min
FIFRA Section 3	Klorkleen 2	71847-7	Medentech Ltd.	Sodium dichloro-s-triazinetrione	30 min
FIFRA Section 3	Accel Concentrate	74559-4	Virox Technologies Inc.	Hydrogen Peroxide	5 min
FIFRA Section 18	various	n/a	various	Sodium Hypochlorite	15 min non-porous; 30 min porous
FIFRA Section 18	various	n/a	Archer Daniels Midland Co	Citric Acid	15 min non-porous; 30 min porous
FIFRA Section 18	Benefect Botanical Daily Cleaner	84683-3	Cleanwell,LLC	Thymol	15 min

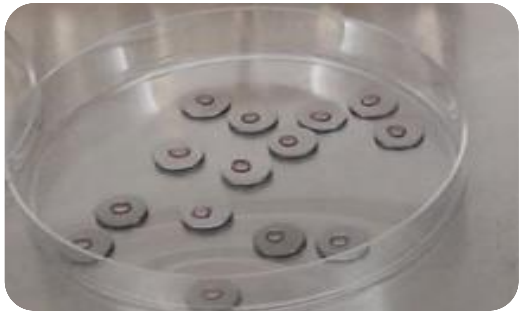
Determining Virucidal Efficacy: Quantitative Carrier Testing

Virucidal Efficacy Test: a quantitative test used to determine the efficacy of a test chemical (chemical disinfectant) for the ability to inactivate a virus. Test methods utilize non-porous coupons (stainless steel/glass) with virus dried in a soil load, or may be based on liquid suspensions (virus not dried). Products deemed efficacious must demonstrate the ability to kill 4 $\log_{10}$ (99.99%) of virus.

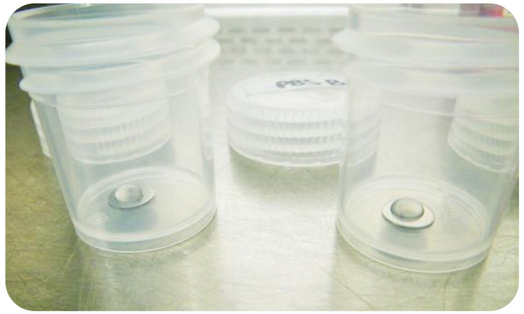
Test Methods

Variable	OECD	ASTM 1053	EN 14675
Virus Volume	10 µl	200 µl	1 mL
Disinfectant Volume	50 µl	2 mL	8 mL
Ratio	1:5	1:10	1:8
Soil Load	32% v/v bovine mucin, FBS, and yeast extract solution	5% FBS	5% FBS
Test Type	Carrier Test (1 cm stainless steel coupon)	Carrier Test (100mm glass petri Dish)	Suspension Test
Water	400 ppm $CaCO_3$	400 ppm $CaCO_3$	400 ppm $CaCO_3$

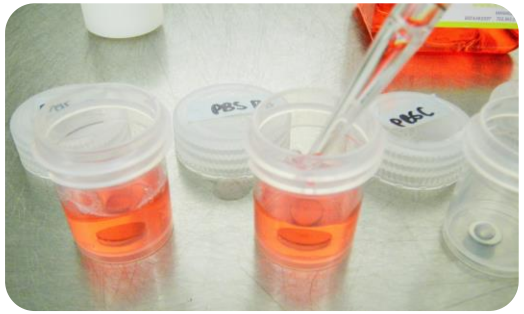
OECD Quantitative Carrier Test



Dry 10 µl viral inoculum (~1 Hr)



Apply 50 µl disinfectant (allow contact time)



Add 10 mL neutralizer and elute virus

→ Titration of ASF BA71V on Vero cells for $TCID_{50}$ determination

Pros:

-Small virus volume decreases inoculum dry time

-Large neutralizer (10mL) to disinfectant (50µl) ratio decreases cytotoxicity to host cell lines

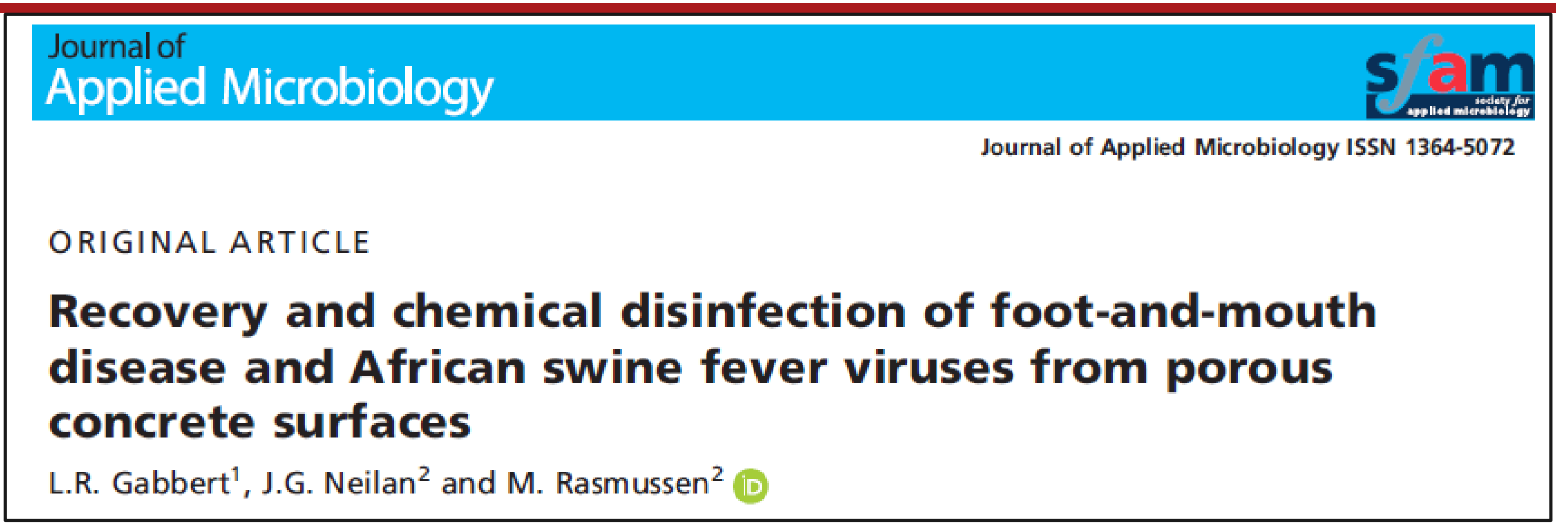
-Standardized 3-part soil load decreases variability

-Multiple replicate coupons

Cons:

-High titer virus stock is needed; may require concentration

OECD Method Development: Porous Surface Disinfection



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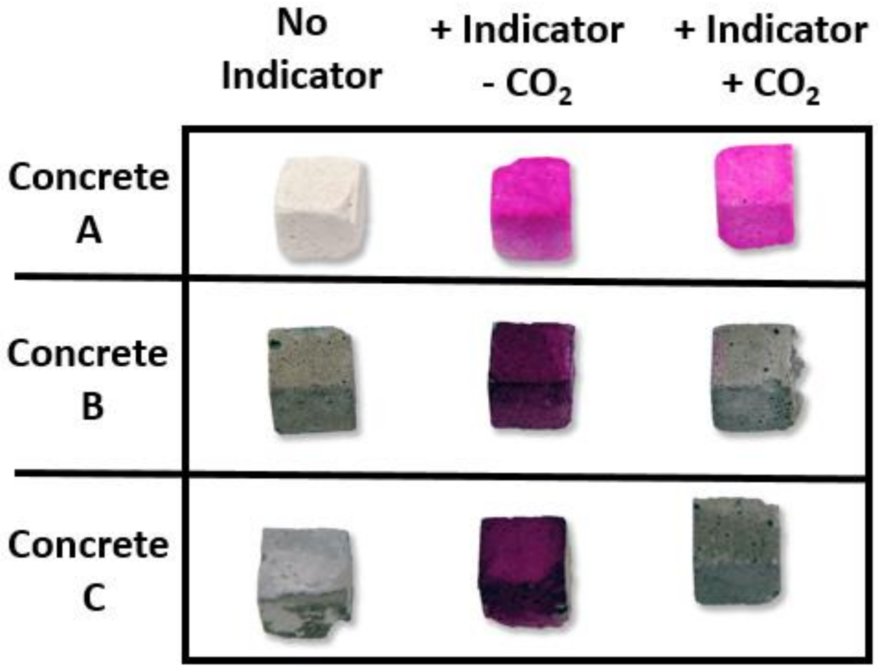
ORIGINAL ARTICLE

Recovery and chemical disinfection of foot-and-mouth disease and African swine fever viruses from porous concrete surfaces

L.R. Gabbert[1], J.G. Neilan[2] and M. Rasmussen[2]

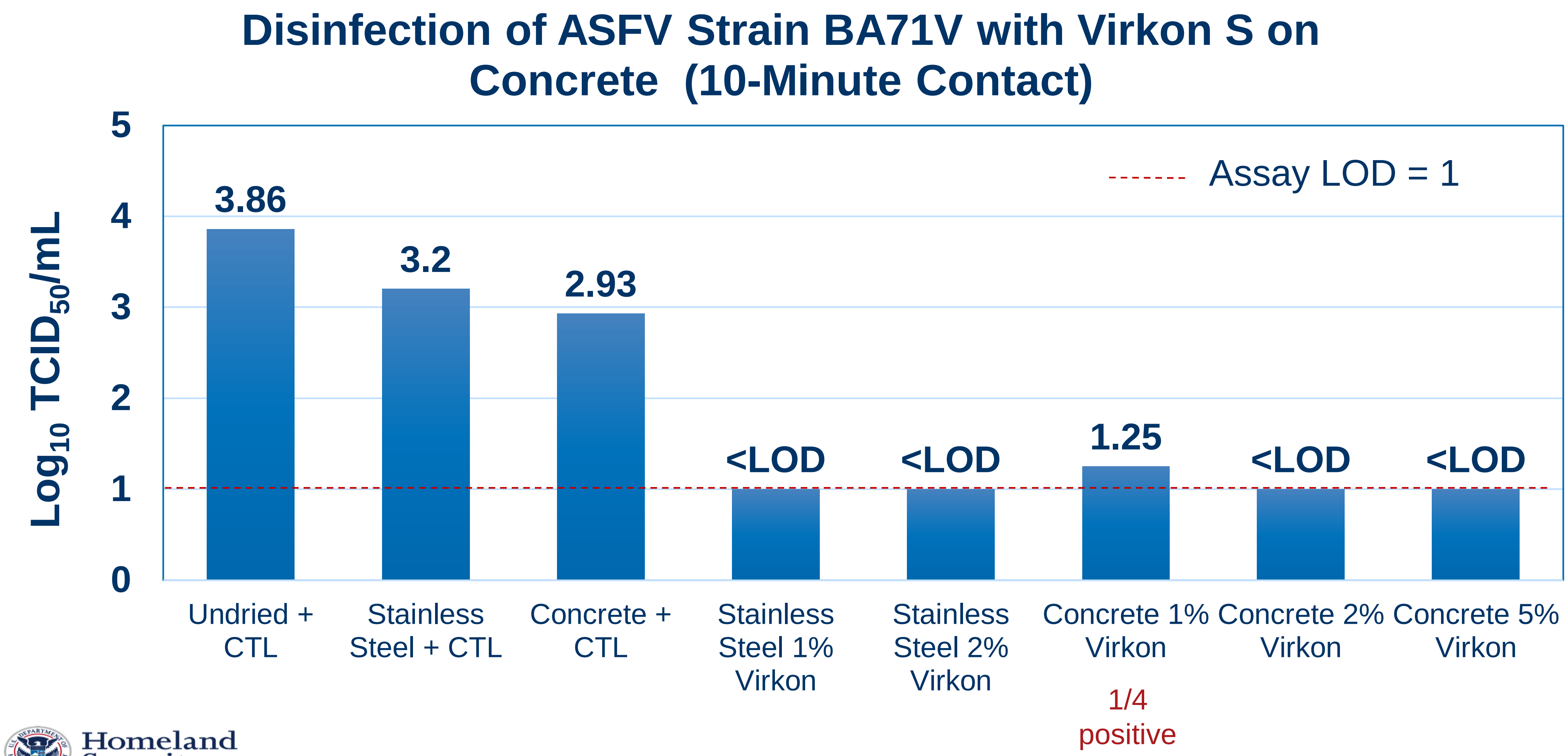
- **Problem:** the high pH of concrete resulted in low recovery of virus from untreated surfaces

- **Solution:** rapid carbonation of concrete coupons via exposure to 5% CO_2 neutralized the pH and allowed for virus recovery from control coupons, thus allowing for further virucidal efficacy testing

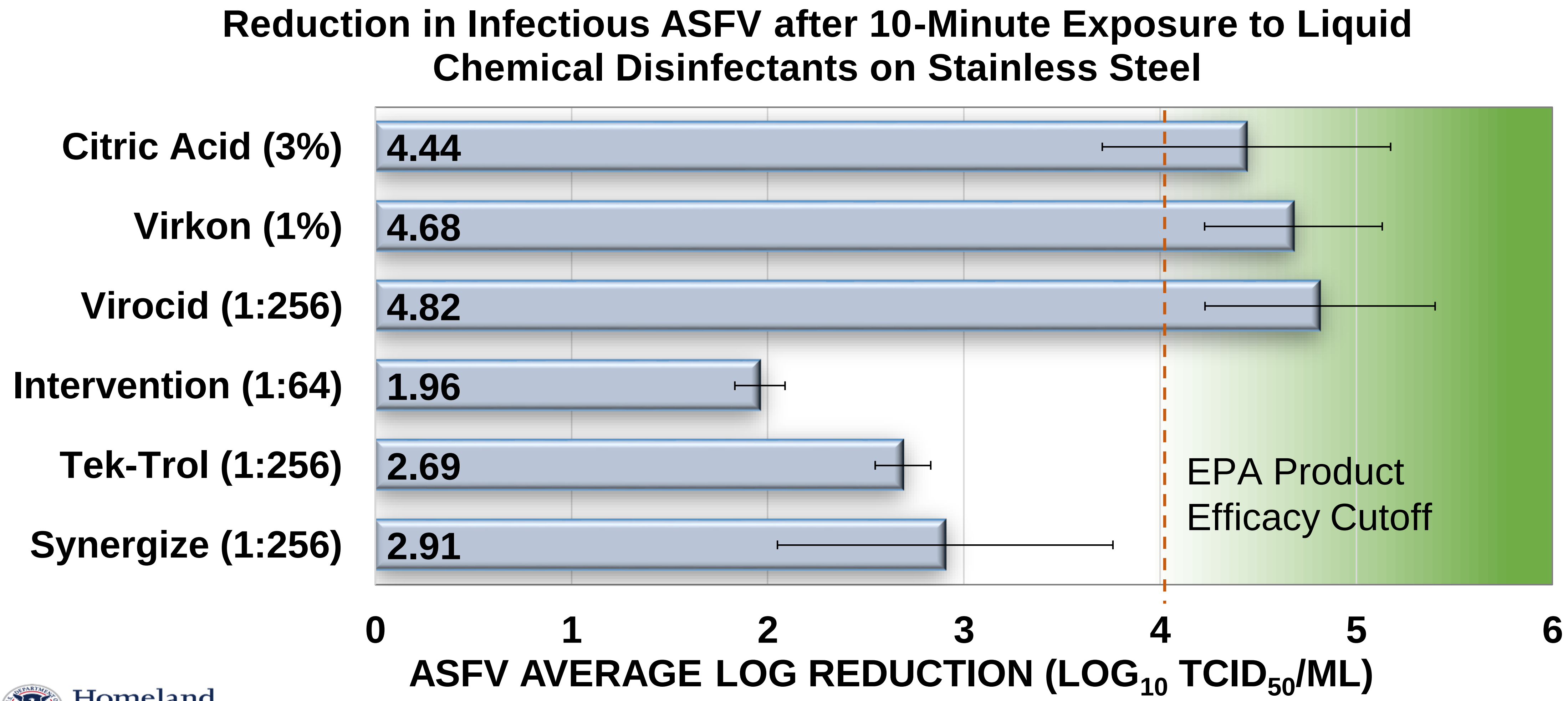


Concrete test coupons with phenolphthalein pH indicator pre- and post-carbonation

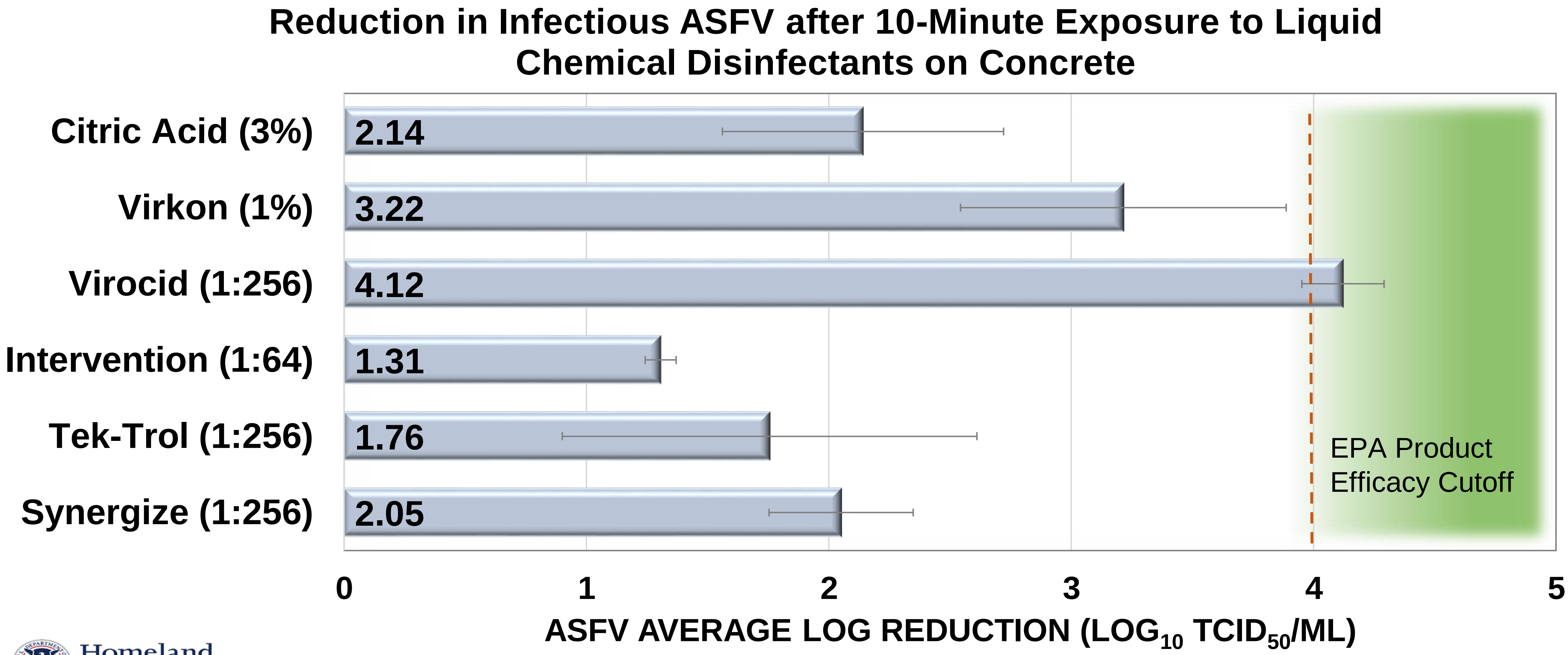
ASFV Disinfection Efficacy Testing: Virkon™ S



Stainless Steel - Summary of Efficacy of Disinfectants Used by Swine Industry



Concrete - Summary of Efficacy of Disinfectants Used by Swine Industry



Summary

- Quantitative carrier tests have proven useful for rapid and repeatable determination of virucidal efficacy of liquid disinfectants against transboundary animal disease pathogens.
- Lessons learned for ASFV disinfection
  - ASFV does not always follow standard disinfection hierarchy rules (more resistant than small non-enveloped viruses in some cases)
  - Chemical formulation is important. Products with similar active ingredients can vary in their ability to inactivate ASFV.
  - Disinfection on porous concrete is possible, but may require higher product concentrations and/or longer contact times.

References

- Blome, S., Franzke, K., Beer, M. (2020). *African Swine Fever: A review of current knowledge*. Virus Research, 287 (2020) 198099
- Gabbert, L, Neilan, J., Rasmussen, M. 2020. Recovery and Chemical Disinfection of Foot-and-Mouth Disease and African Swine Fever Viruses from Porous Concrete Surfaces. Journal of Applied Microbiology. DOI: 10.1111/jam.14694
- OIE. 2019. ASF Technical Disease Fact Sheet. https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/AFRICAN_SWINE_FEVER.pdf

GF-TADs
GLOBAL FRAMEWORK FOR THE PROGRESSIVE CONTROL OF TRANSBOUNDARY ANIMAL DISEASES

Food and Agriculture Organization of the United Nations

WORLD ORGANISATION FOR ANIMAL HEALTH

Thank You