Rapid and Effective Disinfection of Multiple Surfaces Contaminated with Ebola Virus

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Introduction

Emerging infectious diseases (EIDs) represent an ongoing threat to the health and livelihoods of people everywhere. Ebola virus (EBV) is one such EID posing a current threat, with the 2014 outbreak in West Africa responsible for >10,000 deaths and thousands more confirmed cases. EBV is an enveloped virus with a negative sense RNA genome belonging to the family *Filoviridae*. Filovirus virions are unique filamentous particles approximately 80 nm in width and up to 14,000 nm in length. Due to the high mortality rate, EBV is classified as a select agent and handled under BSL-4 laboratory conditions. Infection with EBV results in fever, vomiting, diarrhea and hemorrhage and is transmitted via contact with bodily fluids such as sweat, blood, secretions, saliva, tears, breast milk, stool, nasal drips, and semen. While it is known that EBV particles can persist for long periods of time (weeks) in liquid media of various compositions limited studies have previously been done to assess the persistence of EBV on solid surfaces including porous surfaces and materials with military relevance.

At present there are no U.S. EPA virucidal decontaminants with specific claim labels against EBV. According to CDC and U.S. EPA guidelines, any disinfectant approved for non-enveloped viruses (considered to be more resistant to decontaminants) can be used to disinfect surfaces and objects suspected of EBV contamination. As EBV is a risk group 4 pathogen, studies utilizing EBV are hazardous, difficult and can only be done at a limited number of labs with proper containment facilities. No good surrogate for studying EBV exists due to the unique morphology of the virus and the lack of any closely related viruses of a lower risk level.

Vaccinia virus (VACV), a close relative of the virus that causes smallpox is an enveloped double-stranded DNA virus. VACV possesses a number of attractive characteristics for use as a surrogate for decontamination studies. VACV can be safely worked with under BSL-2 conditions, can be grown to high titers and can be easily quantified by plaque assay. Further, VACV (and other poxviruses) are one of the most persistent viruses on record with live virus recovered after years (at least 13) of storage at room temperature. This study aims to: 1) examine the persistence of EBV/VACV on surfaces of military relevance; 2) assess the efficacy of decontaminants against EBV on these surfaces; and 3) determine the suitability of using VACV as a surrogate for EBV decontamination studies.

Materials and Methods

Viruses -

- Vaccinia Virus (VACV; strain Lister)
- Ebola Virus (EBV; strain Zaire)
- Crude preps in the presence of 10% serum tested
- Initial challenge levels 7-logs (VACV) and 5-6-logs (EBV)

Test Surfaces/Commercial-off-the Shelf (COTS) -

- Steel, TIS (Transport Isolation System), Anti-skid, and Nylon webbing
- 0.5% Bleach, 1% hydrogen peroxide, distilled white vinegar, 1% Bioxy-S, CALLA 1452 (Zip-Chem)

Test Method -

- Standardized OECD Test Method; 30 minute contact time with disinfectant
- The number of viral plaques after disinfectant treatment was compared to untreated controls for each surface type.
- Efficacy expressed as log reduction [Logs plaques (control) – Logs plaques(treated)]

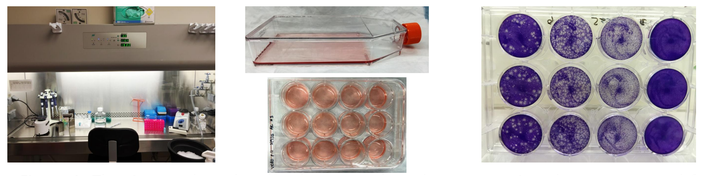
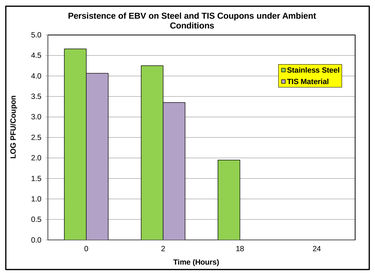
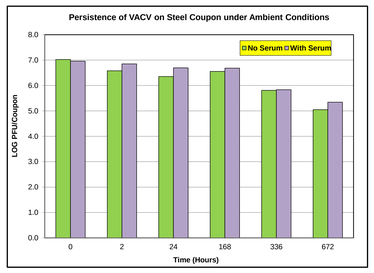
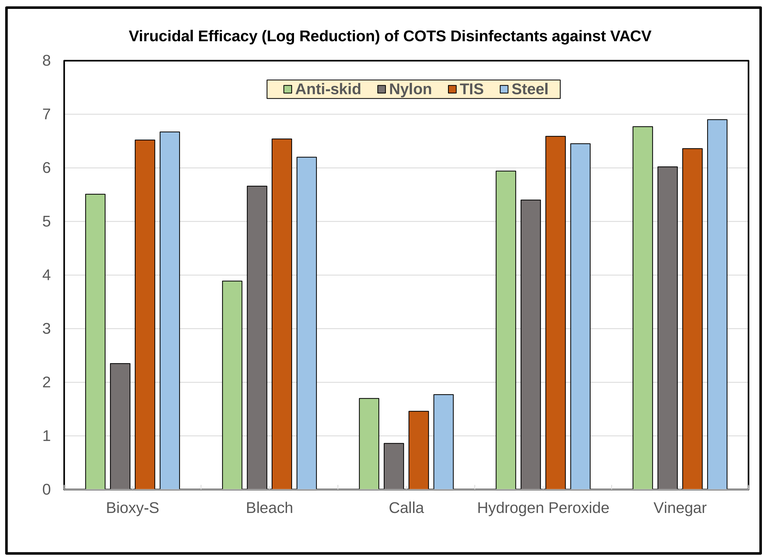


Figure 1. The tissue culture hood, culture vessels and representative plaque assay used for the VACV study are shown

Results







Results Summary

- **VACV persistent for over a month under ambient conditions on hard non-porous surface, i.e. steel, in contrast to EBV (no detectable infectious particles after 24 hours)**
- **Over 6-logs of VACV recovered from all four surface types, in comparison to 5-logs EBV from surfaces**
- **With the exception of CALLA, all other disinfectant technologies were highly effective in reducing the infectious VACV within a contact period of 30 minutes**
- **Both vinegar and 1% Bioxy-S were highly effective in disinfecting EBV on steel and TIS**
- **Interestingly, CALLA is fully effective against EBV within a 30 minute contact period**
- **Vinegar and 1% Bioxy-S are both fully effective in reducing the number of infectious VACV particles within a 5 minute contact period**
- **Tests with a 5 minute contact against EBV are underway**

Results and Discussion

- ✓ The selected test method, OECD, has proven to be a suitable quantitative test method for launching viral disinfection efficacy studies
- ✓ VACV - a large double-stranded DNA enveloped virus, belonging to poxvirus – was found to be highly persistent under ambient conditions (20-25 °C and 25-40% RH)
- ✓ In contrast, EBV – an elongated filamentous virus - was not detectable after 24 hours of drying in the same drying regimes
- ✓ Vinegar and 1% Bioxy-S were both found to be very efficacious in inactivating VACV and EBV, resulting in rapid disinfection of tested surfaces
- ✓ Interestingly Calla (EPA registered) was found to be ineffective in inactivating VACV, but it was found to be highly effective in inactivating EBV
- ✓ Even though, there is no genetic relationship between the two viruses, VACV is determined to be an appropriate surrogate for use in efficacy studies

Conclusions and Future Directions

Future work should focus on:

- ✓ Efficacy evaluation in the presence of body fluids, especially blood
- ✓ Evaluate other enveloped and non-enveloped surrogates for EBV
- ✓ Extend the efficacy study to other surfaces, especially porous fibrous materials, relevant to aircraft and other transportation modes
- ✓ Generate additional robust set of data with rapidly acting disinfectants, such as vinegar and 1% Bioxy-S (non-corrosive and user-friendly)