A Study of the Effectiveness of the Containment Level-4 (CL-4) Chemical Shower in Decontaminating Dover Positive-Pressure Suits

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Abstract

Objectives: The decontamination or physical removal of contaminants from personal protective equipment upon exit from high containment laboratories is crucial to maintain containment and safety of laboratory workers. The current methodology for exiting from high-containment level 4 (CL-4) laboratory calls for laboratory personnel to decontaminate their worn positive pressure suits via “mechanical washing” during a 5-minute chemical/water rinse cycle. This study was carried out to evaluate the effectiveness of the current shower methodology and to test the efficacy of the physical removal of any contaminants from the positive pressure suits following the application of water alone and of a milder detergent.

Method: Brushed stainless steel test carriers were inoculated with Vesicular Stomatitis Virus (VSV), a surrogate for filoviruses, and a soil load based on mucin, tryptone, and bovine serum albumen (BSA) to mimic serum. The virus mixture was then dried and secured with magnets onto a pre-washed Dover positive pressure suit. The suit was subjected to varying shower cycles, and once complete, carriers were removed and further analyzed. Any remaining virus was quantified using a TCID₅₀ assay on Vero E6 cells showing cytopathic effects after 3-5 days. Three positive control carriers with dried inoculum were left untreated by the shower cycle, and negative control carriers (no inoculum) were also included on the suit during shower testing and analyzed alongside the test carriers.

Results: No residual virus was detected using the TCID₅₀ method following the normal shower cycle (2-minute chemical wash and 3-minute water rinse). Shower cycles consisting of 5 minutes of water-only rinses were equally as effective in removing virus from the suit. Cycles consisting of only a 1-minute water rinse showed a 5.5 log decrease from an average initial 6 log viral concentration. Varying time points between 1 and 5 minutes were evaluated during the normal shower cycle as well as varying concentrations of chemical disinfectant.

Conclusion: The current chemical shower protocol is effective in removing all virus from the Dover positive pressure suits worn in high-containment laboratories. However, data show that reducing the need for a high-level decontamination chemical and using any surfactant carries the same effectiveness as the current protocol used, which would serve as a more cost-effective and eco-friendly alternative to the currently implemented shower protocol.

Introduction

The study of infectious agents within a maximum containment laboratory often poses unique challenges for the personnel who work within the laboratory and the engineers who design the laboratory space. Special attention must be paid to the laboratory design to ensure a safe and secure environment in which scientists and technicians may conduct their work with high risk group organisms. Concern should also be granted to the surrounding area and community to decrease the risk of potential release of an infectious agent, through either accidental or intentional means. In particular, the decontamination of personal protective equipment (PPE) upon exit from containment laboratories must be adequate to significantly reduce the risk of release to the environment. The 1978 accidental release of Foot-and-Mouth Disease (FMD) virus on Plum Island (NY) resulted in the infection of many animals outside of the containment area, but restricted to the island (Rathé, 2004). This incident and the FMD release from Pirbright in 2007 illustrate the dangerous effects of an unintentional release of a USDA select agent (Rhodes, 2009). Although these examples highlight an animal virus release and the economic consequences, it still serves to demonstrate the potential devastation that could result from the release of an infectious agent affecting humans.

According to the Biosafety in Microbiological and Biomedical Laboratories (BMBL) (5th edition) manual (2009), organisms studied within a containment level-4 (CL-4) laboratory are defined as “dangerous or exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections and for which there is no available vaccine or therapy; or related agents with unknown risk of transmission” (U.S. Department of Health and Human Services, 2009). Haemorrhagic fever viruses (HFVs) fall under this category and their clinical disease manifests itself directly as severe internal haemorrhaging and fever (Bosi et al., 2004). The most common HFVs are lipid-enveloped RNA viruses belonging to the family Filoviridae, Arenaviridae, Flaviviridae, or Bunyaviridae and are capable of aerosol transmission and infection in humans and animals, although most transmission occurs through direct contact with animal rese-
voirs or arthropod vectors (Bossi et al., 2004). High mor-

bidity and mortality rates are often observed in popula-

tions infected by these viruses, which may increase their

likelihood to be intentionally released (Bossi et al., 2004).

Infectious agents pose a high risk to both the indi-

vidual and community, necessitating the use of good

laboratory practice and technique, safety equipment and

PPE (primary barriers), and facility design (secondary

barrier) for safe and proper functioning of high-

containment research and clinical laboratories (U.S.

Department of Health and Human Services, 2009). Such

safety equipment includes the use of biological safety

cabinets (BSCs) in conjunction with a fully-contained,

air-supplied positive pressure suit worn by personnel when

conducting any procedures within the CL-4 lab space

(U.S. Department of Health and Human Services, 2009).

Subsequently, upon exit from a CL-4 laboratory, person-

nel are required to decontaminate the worn positive

pressure suits via a chemical shower to maintain con-

tainment, personnel safety and to prevent accidental

release of dangerous biological agents into the environ-

ment and surrounding community.

The current guidelines in Canada, as prescribed by

the Laboratory Biosafety Guidelines (3rd edition) (2004),

require a “chemical shower of appropriate duration for

personnel in suits who are leaving the containment

laboratory; the disinfectant used must be effective against

the agents of concern, be diluted as specified and pre-

pared fresh as required” (Public Health Agency of Cana-

da, 2004). No specification is given to the class or con-

centration of disinfectant to be used or to the total dura-

tion of the chemical shower. Currently, only limited data

case the overall effectiveness of the chemical shower in

properly removing biological agents. Some North Ameri-

can containment facilities have adopted a protocol consist-

ing of a 2-minute 5% MicroChem-Plus™ (National Che-

mical Laboratories, Inc., Philadelphia, PA) wash, followed by a 3-minute rinse with water. Proper

showering procedure requires personnel to mechanically

remove contaminants from arms, legs, shoulders, head, etc. to maximize the effectiveness of the decontaminant

wash cycle to physically remove any residual agent(s)

from the positive pressure suits (National Microbiology

Laboratory, 2005). All effluent waste water is collected in

storage tanks and heat-inactivated before its final

discharge.

The purpose of this study was to evaluate the effec-

tiveness of currently implemented chemical shower pro-

cedures to remove residual virus from the positive pres-

sure suits upon exit from the CL-4 laboratory. The effec-

tiveness of a pure water rinse cycle and of a mild surfac-

tant wash in combination with a water rinse cycle was

also tested to determine their decontamination efficacy.

Each shower cycle was tested at 1-, 3-, and 5-minute

exposure times, using Vesicular Stomatitis Virus (VSV) as

a surrogate for level-4 filoviruses. Brushed stainless steel

carriers were inoculated with an organic soil load and

VSV, affixed onto the positive-pressure suit and exposed
to the detergent (or water) in the chemical shower area.

For this study, VSV is used as a surrogate for other

commonly encountered CL-4 viruses. Although it is found

in the Rhabdoviridae family of arboviruses, it shares

many similar characteristics with the HFVs studied within

the CL-4 facility (Kuzmin et al., 2009). The Rhabdovi-

ridae family belongs to the order Mononegavirales which

also includes such families such as Bornoviridae, Filovi-

ridae, and Paramyxoviridae based on their shared mor-

phological characteristics (Kuzmin et al., 2009). VSV

contains a lipid bilayer envelope surrounding its helical

nucleocapsid which encloses negative, single-stranded

RNA (Kuzmin et al., 2009).

MicroChem-Plus™ is the quaternary ammonium

compound used within CL-4 chemical showers (at a 5%
n Concentration) to decontaminate the positive-pressure

suits worn within the containment lab space. It is a catic-
onic surfactant effective against vegetative bacteria,

fungi, and enveloped viruses, which binds to the anionic

outer membrane components of these agents, solubili-

zing the lipid membranes and allowing for their subse-

quent removal from surfaces (Gilbert & Moore, 2005).

The active compounds include 2.25% of alkyl dimethyl

benyl ammonium chlorides and 2.25% of alkyl dimethyl

ethybenzyl ammonium chlorides, both with low biodegra-

dability, lending to their potential accumulation in the

environment (World Health Organization, 2004).

A mild detergent was also tested to determine its

decontamination efficacy. In this study, a 5% solution of

Dove Moisturizing Gentle Hand Cleaner™ (Johnson Di-

versey, Sturtevant, WI) was chosen, with the main sur-

cfactant ingredients being ammonium laureth sulphate

and sodium cocyl isethionate. The solution is also made

up of cocoamidopropylbetaine, which contains a quater-

nary ammonium cation, conferring mild antiseptic prop-

eries to the detergent.

Materials and Methods

Preparation of VSV Stock

Vesicular Stomatitis Virus (Indiana strain) was inoc-

ulated onto a monolayer of Vero E6 cells in 2% FBS

DMEM (Dulbecco’s Modified Eagles Medium, GIBCO) at

a multiplicity of infection of 0.1 and allowed to replicate

for 48 hours. The medium was then centrifuged at

4,000 × g and the supernatant was collected, resus-

pended in DMEM, and aliquoted into 200 µL volumes to

be stored at -80°C.

Neutralization Solutions

Dey-Engly (DE) neutralization solution (DIFCO) at a

1:1 ratio of 2x DE broth:2x DMEM was used to neutralize

5% MicroChem-Plus™ following a 1-minute exposure to

the decontaminant (neutralization assay results shown
in Figure 1). A plain solution of DMEM was used to neutralize all other chemical and water treatments at each exposure time.

Inoculation of Brushed Stainless Steel Carriers

Brushed stainless steel discs were prepared according to the first steps of the Quantitative Carrier Test Tier-2 (QCT-2) (Sattar & Springthorpe, 2003) within a Class II biosafety cabinet (BSC) in a CL-2 lab. VSV (at a concentration of about 10^6) and soil loads (5% BSA, 7% tryptone, and 20% mucin) were inoculated onto carrier discs and allowed to dry for 45-60 minutes in a Class II BSC. One negative control (disc with no inoculum) was tested alongside the inoculated carriers. Three carriers served as positive controls (not subjected to chemical or water exposure) and were used to calculate the overall starting concentration of VSV. Dried discs were then transported to the CL-4 chemical shower to be affixed onto the Dover positive-pressure suit.

CL-4 Chemical Shower Testing

High-powered circular magnets (7 mm in diameter) were taped onto the inside of a pre-washed Dover positive pressure suit at nine predetermined areas: back, left arm, right arm, right chest, right thigh, left thigh, right hip, left hip, and right boot cuff (Figure 2). The carrier placed on the back was used to mimic a “soak washing” procedure. The negative control carrier was placed at the boot cuff area.

Inside the chemical shower area, carriers were aseptically affixed onto the suit and exposed either to the facility’s standard chemical shower procedure, a rinse cycle consisting solely of water, or a mild surfac- tant cycle at three time points: 1 minute, 3 minutes, and 5 minutes (comprising a complete cycle). Each time point was tested in triplicate independently of the others. Table 1 outlines the duration and composition of the various shower cycles tested.

Following exposure to the various treatments, each carrier disc was aseptically removed from the suit and placed into Teflon vials containing 1 mL of either DE neutralization solution or plain DMEM. Vials containing the exposed carriers were removed from the CL-4 area through the personnel shower and further processed in a Class II BSC in a CL-2 lab.

Tissue Culture Infectious Dose 50 Quantification

Exposed carriers were flushed and scraped with the pipette tip for 45-60 seconds to elute any remaining virus (also performed on positive control carriers that were previously prepared).

Ten-fold serial dilutions (10^7-10^7) of eluate were performed in 10% FBS DMEM, and 50 μL of each dilution were transferred (in quintuplicate) to a monolayer of 90% confluent Vero E6 cells in 96-well plates and incubated for 60 minutes at 37°C to allow for viral adsorption. Following incubation, wells were diluted to achieve a 2% FBS DMEM complete media concentration and incubated for 2-3 days at 37°C. Cytopathic effect (CPE) development in cells was observed, and the presence and concentration of remaining virus were determined via TCID_{50} calculations.

Data Analysis

Tissue Culture Infectious Dose-50 (TCID_{50}) is defined as the amount of virus necessary to cause infection in

![Figure 1](image-url)

Neutralization control assay results on Vero E6 cells at 90% confluency and 3-5 day incubation at 37°C.
Figure 2
Diagrammatic representation of carrier placement on Dover positive pressure suit during chemical shower efficacy testing; a) front view of carrier placement; b) back view of carrier placement.

Table 1

<table>
<thead>
<tr>
<th>Shower Cycle</th>
<th>Time Point</th>
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<tbody>
<tr>
<td></td>
<td>1 minute</td>
</tr>
<tr>
<td>Standard Chemical Shower</td>
<td>1-minute 5%</td>
</tr>
<tr>
<td>Water Rinse</td>
<td>1-minute water rinse</td>
</tr>
<tr>
<td>Mild Surfactant Shower</td>
<td>1-minute 5%</td>
</tr>
<tr>
<td></td>
<td>Dove™ detergent</td>
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<tr>
<td></td>
<td>1-minute water rinse</td>
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50% of inoculated cells. The proportionate distance, or the interpolated value of the 50% endpoint, is calculated and then multiplied by the logarithm of the last dilution factor showing CPE above 50% of test units. The logarithm of this value is then calculated to obtain the endpoint titer per milliliter.

Average starting concentrations of VSV were determined by taking the average of all log TCID₅₀ calculations of the positive test carriers (no exposure to treatment) of each particular shower cycle. Three separate starting concentrations of the standard shower cycle, water rinse cycle, and Dove surfactant cycle were obtained.

Results
Overall average starting concentrations (measured as log TCID₅₀/carryer) of VSV for the standard chemical shower procedure, water rinse cycle, and Dove surfactant cycles were 6.26±0.65, 5.96±0.55, and 6.09±0.69 respectively (Table 2). At 1-, 3- and 5-minute exposure times, the standard chemical shower procedure was successful in achieving a complete log reduction of the average initial starting concentration of VSV. The water rinse cycle, however, was able to achieve a complete log reduction of the initial starting concentration only at the
5-minute exposure time. At the 1-minute exposure time, a 5.5 log reduction was observed, and at the 3-minute exposure time, a 5.8 log reduction was achieved. The Dove surfactant cycle at 3- and 5-minute intervals was also able to achieve a complete log reduction in the initial VSV concentration. The 1-minute exposure time achieved a 5.9 log reduction of total average starting VSV concentration (Figure 3).

All shower cycles achieved a complete 6 log reduction in VSV concentration at the 5-minute exposure time (Figure 3).

Discussion

Results show that all decontamination shower cycles were successful in completely removing all infectious virus from the Dover positive pressure suit at a 5-minute exposure time. This indicates that the use of a surfactant may not be necessary to achieve complete elimination of VSV particles during the decontamination shower.

Greatest variability in results occurred at the 1-minute exposure time. The water rinse cycle showed 0.52±1.03 logs of remaining VSV on the carriers after a 1-minute exposure time. Distribution of remaining virus on the test carriers was seemingly random: 2 logs on the back, 2.3 logs on the right leg, 2.8 logs remaining on the left thigh, and 2.5 logs remaining on the left hip, which in turn attributed to the large standard deviation in results at that particular exposure time. The right arm and right hip areas both showed positive CPE in one test unit each, but due to the limitations of the TCID\textsubscript{50} analysis, the remaining virus concentration at these particular carriers was below the detectable threshold at the lowest dilution. In this case, results were reported as no remaining virus on these carriers.

The mild surfactant cycle also showed some residual VSV (1.8 logs) following a 1-minute exposure time, but only on the carrier placed on the right chest area. All other carriers exposed during this cycle showed no evidence of remaining VSV following TCID\textsubscript{50} analysis (also attributing to its relatively large standard deviation). This may have been due to the physical spraying effect of the Dove decontaminant, which was done manually using a single pressurized canister. Uneven spray distribution

### Table 2

<table>
<thead>
<tr>
<th>Shower Cycle</th>
<th>Average Log TCID\textsubscript{50}/Carrier</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Exposure Time</td>
</tr>
<tr>
<td></td>
<td>0 minutes</td>
</tr>
<tr>
<td>Standard Chemical Shower Procedure</td>
<td>6.26±0.65</td>
</tr>
<tr>
<td>Water Rinse Cycle</td>
<td>5.95±0.55</td>
</tr>
<tr>
<td>Mild Surfactant Cycle</td>
<td>6.09±0.69</td>
</tr>
</tbody>
</table>

### Figure 3

Average log TCID\textsubscript{50}/carrier reduction of VSV after 1-, 3-, and 5-minute exposures of the standard chemical shower procedure, water rinse cycle, and dove surfactant shower cycle.
during this short exposure time may have been compensated for during the longer exposure times, thus showing greater log reduction in starting viral concentrations. The standard chemical shower cycle achieves an overall larger spray distribution area, as both chemical and water are sprayed from all four corners of the shower room and at a greater psi than is generated using a single pressurized spray canister.

The DE neutralization solution was used only for the 1-minute standard chemical shower cycle as there may have been residual MicroChem-Plus™ left on the carrier surface following exposures, necessitating the use of a neutralizer. For all other cycle time points, plain DMEM was used, as the water rinse would have removed any remaining decontaminant.

Cytotoxicity testing showed that the DE neutralization solution and 5% MicroChem-Plus™ mixture were cytotoxic to Vero E6 cells (Figure 1). To ensure no false-positive CPE development during actual shower testing and analysis, 500 μL of the eluate was diluted in 5 mL of 2% FBS DMEM solution on a monolayer of 90% confluent Vero E6 cells. No CPE development was observed following a 3- to 5-day incubation at 37°C, confirming that the CPE formed was solely due to the cytotoxicity of the neutralizer solution and no infectious virus was present.

**Conclusion**

From the results obtained, it is evident that the standard shower cycle procedure currently implemented is effective in removing all VSV from the Dover positive pressure suit. The milder surfactant chemical cycle was equally effective in removing virus at the 3-minute exposure time. At the 5-minute exposure time, the pure water rinse was completely effective in eliminating all infectious VSV from the stainless steel carriers. Use of a milder surfactant in lieu of a harsh quaternary ammonium compound with low biodegradability during a complete chemical shower cycle is just as effective in removing lipid-enveloped virus from the positive pressure suit. As the overall decontamination process is based on the effective mechanical removal of contaminants, variability in results may be largely dependent on the efficacy of each rub-washing procedure in which an individual may engage. It is evident that the effectiveness of the chemical shower in decontaminating the Dover suits resides not in the potency of the chemical used, but rather in the proper training and technique implemented during the decontamination procedure. This could potentially decrease the dependency on costly and environmentally hazardous chemicals to successfully decontaminate PPE upon exit from a maximum-containment laboratory, as was clearly demonstrated by this study.

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**References**


