A Bacteriophage-based Validation of a Personal Protective Equipment Doffing Procedure to be Used with High Consequence Pathogens

Running Title: A Bacteriophage-based Validation of PPE

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Abstract

Objective To determine if the high-level personal protective equipment used in the treatment of high consequence infectious diseases is effective at stopping the spread of pathogens to healthcare personnel (HCP) while doffing.

Background Personal protective equipment (PPE) is fundamental to the safety of HCPs. HCPs treating patients with high-consequence infectious diseases use several layers of PPE, forming complex protective ensembles. With high-containment PPE, step-by-step procedures are often used for donning and doffing to minimize contamination risk to the HCP, but these procedures are rarely empirically validated and instead rely on following infection prevention best practices.

Methods A doffing protocol video for a high-containment PPE ensemble was evaluated to determine potential contamination pathways. These potential pathways were tested using fluorescence and genetically marked bacteriophages.

Results The experiments revealed existing protocols permit contamination pathways allowing for transmission of bacteriophages to HCPs. Updates to the doffing protocols were generated based on the discovered contamination

pathways. This updated doffing protocol eliminated the movement of viable bacteriophages from the outside of the PPE to the skin of the HCP.

Conclusions Our results illustrate the need for quantitative, scientific investigations of infection prevention practices, such as doffing PPE.

INTRODUCTION

To protect healthcare personnel (HCPs) caring for patients with communicable diseases, protocols have been established to mitigate the risk of transmission [1]. Central to these protocols is personal protective equipment (PPE). The PPE used to minimize exposure to high consequence infectious diseases (HCIDs), such as Ebola virus disease, utilizes layers of barrier precautions including fluid-resistant coveralls, impervious aprons or gowns, fluid-resistant footwear, powered air-purifying respirators (PAPRs), and gloves. Protocols that outline proper donning and doffing of the PPE are fundamental to mitigating self-contamination for HCPs and preventing the transmission of contaminants outside patient rooms [2]. However, PPE protocols are often based on manufacturer recommendations of individual products and infection prevention best practices. Accordingly, ensembles of PPE and their corresponding protocols usually have not been empirically validated.

Previous studies have demonstrated that adherence to PPE doffing protocols is challenging and variable among HCPs [2]; previous studies have quantitatively examined and discovered high rates of deviations from established protocols [3]. Doffing protocols would optimally be safe even considering this high underlying

47 variability. Moreover, rigorous risk assessments of PPE ensembles should
48 consider this factor in evaluating PPE safety.
49
50 This study explores contamination risks of an established doffing protocol. To
51 validate this protocol's efficacy, we applied a quantitative analysis of the PPE
52 ensemble to test for self-contamination. This investigation consisted of two
53 phases: 1) examination of the original PPE ensemble and its doffing protocol with
54 fluorescence and bacteriophages; and 2) determination if an amended protocol
55 decreased self-contamination.
56
57 This study highlights the need for infection prevention protocols, such as high-
58 containment PPE doffing, to be evaluated in a quantitative, experimental fashion.
59 We present results from both phases of our investigation below.
60

METHODS

Investigational Procedures

Movie analysis

Doffing protocols were captured on video. Each movie was analyzed by authors BAB, KBB, APS, and JSM who viewed and stopped the movie and recorded comments when potential hazards were observed.

For each doffing trial, footage was taken from four camera angles using camcorders (Canon, Japan, Model #HF R80) supplied by the Healthcare and Human Factors Lab at Emory. Comments were made for possible contamination during the Phase 1 phage trial and deviations from the protocol in the Phase 2 phage trial (Supplementary Table 1).

Fluorescent testing

To verify the contact we observed in the movie described above, we used a 0.5% fluorescein solution (Millipore Sigma, USA, Catalogue #F6377) and Glo Germ powder (Glo Germ, USA, #GGP10) to visualize contamination and identify the steps where spread of contaminant may occur. To conduct this test, a trained HCP donned a complete high-containment PPE ensemble and was then sprayed with the fluorescein solution. The solution was sprayed to coat surfaces of the PPE that may be exposed to contact with patients (patient-facing surfaces). The

HCP then doffed according to the protocol. Pictures were taken from multiple angles to record how fluorescein transfer; ultraviolet flood lights (Onforu, China, #UFLAU004102) were used to emphasize fluorescence. A separate test was performed for aerosolization risk where the heavy-loading filter on top of the PAPR hood was laden with Glo Germ powder. After the filter was removed, pictures were taken with ultraviolet to capture results of the experiment.

Phage testing

Using a previously validated procedure [4], we sprayed with small spray bottles (Bürkle, Germany, #10216-888) high densities (approximately 10^8 phages per mL) of three genetically distinct λ phages on HCP volunteers to reflect densities of pathogens found in patient samples (Supplementary Table 2). 2mL of λ phages were sprayed onto three locations, and each site was sprayed with a different variant of λ: one marked with a kanamycin resistance gene was sprayed on the wrists; one marked with a chloramphenicol resistance gene was sprayed on the back of the hood; and one lacking an antibiotic marker at the critical triangle, described below. HCPs then doffed, after which their hands, forearms, and PAPR were swabbed and scrubs were collected and tested for bacteriophage presence and identification.

Materials and Technical Methods

Strains

106 *Escherichia coli* strain C was acquired from Marie-Agnès Petit from INRAe,
107 France. Bacteriophages λ (λ^{Temp}), λ^{Chl}, and λ^{Kan} was obtained from Maroš Pleška
108 at The Rockefeller University.
109
110 *Bacteriophage Lysate Preparation and Distribution*
111 Each type of λ phage lysate were inoculated, shaken, centrifuged, and filtered
112 per the methods in Burke et al. [4] to create high-titer lysates (PFU/mL between
113 $1x10^8$ and $1x10^9$). These lysates were stored in spray bottles, transported, and
114 primed per the methods in Burke et al. [4]. Immediately after priming, each lysate
115 was sprayed with one pump from a distance of 10 cm onto the target sites for
116 initial contamination. The spray dried clear and were unidentifiable to the naked
117 eye. Contamination occurred no earlier than five minutes before the start of the
118 doffing.
119
120 *Bacteriophage Recovery*
121 Immediately after the doffing procedure, skin was sampled by applying a saline
122 wipe (Hygea, USA, #C22370) around the hands and a wipe around the forearms;
123 these wipes were stored in conical tubes (Corning, USA, #352070). Disposable
124 scrubs were then stored in a Whirl-Pak (Nasco, USA, #B01542). Four sites of
125 interest were swabbed with self-contained saline swabs (Hardy Diagnostic, USA,
126 #SRK35) using a progressive back-and-forth motion until the entire surface
127 appeared damp. To liberate phage from the saline wipe, the wipe was squeezed
128 to remove excess liquid and the extracted solution was tested. To recover phage

from the scrubs, 300mL of deionized water was added to the bags that contained the scrubs and shaken vigorously to ensure scrubs were fully saturated. Excess liquid was poured into a conical tube for testing. To recover phage from the swabs, the saline containers were vortexed vigorously.

After bacteriophage recovery, all surfaces with possible phages were sprayed with 70% ethanol (Decon Labs, USA, #2716) and wiped with Sani-Cloth Disposable Wipes (Professional Disposables International, Inc., USA, #Q55172).

Bacteriophage Identification and Quantification

Phage identification was performed by PCR, using the methods and materials used in Burke et al. [4]. Band sizes of 800bp were called λ^{Temp}, 1500bp called λ^{Chl}, and 1900bp called λ^{Kan}. The PCR was performed with an O'Gene Ruler DNA Ladder (Thermo Fisher Scientific, USA, #SM1563).

The serum resistance lipoprotein (*bor*) gene (Gene ID: 2703532, NCBI) of the λ phages was amplified by PCR using the following primers designed in PrimerBLAST (NCBI): Forward (borRG1Fw) 5'-GCTCTGCGTGATGATGTTGC-3' and Reverse (borRG1Rv) 5'-GCAGAGAAGTTCCCCGTCAG-3'. Using the double layer soft agar method [5] LB soft agar overlays containing 0.1 mL of a turbid *E. coli* C overnight were prepared and allowed to harden. 0.01 mL of serially diluted saline recovery solution was spotted on the overlay at four

densities. These plates were grown overnight at 37°C, and plaques were enumerated the next day.

If samples were determined to be PCR positive but negative via spot testing, 100µL of sample were cultured with $1x10^7$ CFU/mL log-phase *E. coli* C in 10mL of LB broth. These cultures were grown with shaking for six hours, centrifuged, and filtered through a 0.22 µm filter to generate boosted lysates. 300µL of these lysates were plated on *E. coli* C lawns to determine viable bacteriophage presence.

Process Documentation (Videography and Still Photography)

For the fluorescein and Glo Germ experiments, pictures were taken with an iPhone under ultraviolet illumination in a dark room; footage was recorded with one camcorder. During doffing trials, pictures and footage were recorded under standard room lighting.

RESULTS

Initial protocol analysis

Analysis of initial doffing movie

The investigation of the initial protocol (detailed in Supplementary Table 3, 4, 5) began with a movie analysis. Table 1 notes observations of potential sources of contamination to the HCP during the doffing protocol displayed in Movie 1. Steps can be correlated to the steps of the original protocol in Supplementary Table 3.

Evaluating contamination via fluorescence testing

In Figure 1 we present several pictures of testing with fluorescence that illustrate the concerns raised by the movie. We note that the protocol as performed in our trials, including this one, follows the written protocol and differs slightly from Movie 1; Movie 2 accurately portrays the written doffing protocol. There are six pairs of pictures: The left (L) panes show areas of concern, and the right (R) panes show the spread of fluorescence from those events.

The fluorescein reveals the materials in the complete PPE ensemble that are patient-facing. 1AL shows a complete ensemble, and 1AR shows the ensemble without the apron. 1BL shows the ensemble under blacklight, and 1BR shows contamination not covered by the apron. 1CL and 1CR highlight the “Critical

188 Triangle", which includes parts of the shoulder, the side of the abdomen, and the
189 arm.

190 The movement of the fluorescein demonstrated in Figure 1 reveals how
191 contamination can move from the outside of the PPE ensemble to an
192 intermediate location, then ultimately to the HCP. These contamination pathways
193 are demonstrated in 1D, 1E, and 1F. 1DL shows how contamination may reach
194 the arm or Critical Triangle of the coveralls. From here, contamination could
195 transfer to the underside of the shroud (shown in 1DR) which could then move to
196 scrubs. 1E shows a second pathway, where contamination on a patient-facing
197 shoulder (1EL) transfers to arms when reaching up to roll up the PAPR hood,
198 with that contamination demonstrated in 1ER. 1F demonstrates how aerosolized
199 pathogens land on skin, scrubs, and footwear.

200 Using these results, three initial locations on the PPE were determined to pose a
201 high contamination risk. These locations are (i) the PAPR hood Critical Triangle,
202 located to the left and right of the apron and near shoulders, (ii) the wrist/lower
203 forearm area of the protective coverall, and (iii) the back of the PAPR hood near
204 the filter and shoulders.

205

206 *Doffing in the presence of a bacteriophage proxy*

207 To more accurately mirror pathogenic contamination, we inoculated three
208 genetically marked variants of λ on the three sites above to determine both the

origin and final location of each virus. Presented in Figure 2 are the results of doffing performed by four HCPs with varying heights and body types and varying experience in performing the protocol.

Figure 2 demonstrates that phages moved to the four locations we had hypothesized could become contaminated. Moreover, we found these phages to be viable and present at high densities. All four HCPs demonstrated contamination.

These four HCPs were recorded from multiple angles while performing the doffing procedure. We present in Table 2 behaviors noted during our analysis of the movies which would increase the risk of contamination.

Updated protocol analysis

Changes to the Protocol

Our analysis and experiments of the first protocol revealed insufficiencies that led to contamination of the HCP. We aimed to eliminate viable phage recovery by limiting the observed contamination pathways. Accordingly, we altered the protocol in both equipment and doffing steps (Movie 3; Supplementary Table 3; Supplementary Table 4; Supplementary Table 5). Below is a table detailing changes made to the PPE ensemble and procedure.

Six amendments were made to the protocol. Adjustments were made based largely upon concerns raised by the phase one analysis, but amendments were also incorporated for ease of doffing. Of the six changes, three were changes in equipment; one was an additional step made for added equipment; and two were reordered steps.

Phage Testing with Updated Protocol

We next evaluated the updated protocol with the phage testing described previously. Nine HCPs doffed using the updated protocol. The results of these doffing trials are presented in Figure 3.

Following the updated protocol, no viable phages were recovered. Phage DNA was found via PCR from several locations (indicated by an X) but viable phages were unable to be recovered from these PCR-positive samples even after providing a bacterial host. This indicates that the phages moved during doffing, but these phages were likely inactivated by the alcohol-based sanitizer during hand hygiene. Even if a contamination pathway was not eliminated, the updated protocol limited those pathways to contamination on gloves where sanitation could deactivate the bacteriophages.

Deviations from the doffing protocol by HCPs could contribute to variability in the results shown in Figure 3. We analyzed footage of each HCP doffing and noted deviations from the protocol which may lead to the spread of bacteriophages (Supplementary Table 1). Several HCPs deviated from the procedure. However, these deviations did not increase contamination per the results in Figure 3.

DISCUSSION

PPE forms the cornerstone of safety for HCPs, but for HCPs working with HCIDs, satisfactory high-containment PPE is especially important[6]. Hundreds of HCPs experienced near-miss events, infections, or death from Ebola virus disease [7, 8]. Although individual pieces of equipment receive National Institute for Occupational Safety & Health approval, PPE ensembles and their doffing protocols do not. Indeed, Koh et al. wrote over twenty years ago that PPE needed to be evaluated for efficacy against infection from SARS – this is a problem that has needed addressal for decades [9], and later, the same call to action was issued for empirical review of Ebola PPE and ensembles [10].

This PPE ensemble had not been assessed by empirical means. Our goal was to evaluate the ensemble and doffing protocol for possible contamination pathways and offer interventions to mitigate potential contamination. Even a slight contamination of an HCID could be a threat to HCPs (Supplementary Table 2). Thus, the aim of our interventions was to prevent viable self-contamination. We note that although other methods exist for analyzing the antiviral and disinfection qualities of PPE and its ensembles [11, 12], we elected to focus exclusively on how this ensemble performed in the transfer of pathogens.

Our first phase of this study began with an examination of a movie depicting the original protocol. The original protocol followed infection prevention best practices and was designed with disease containment in mind, but an in-depth evaluation revealed potential contamination pathways. We found from the fluorescence testing that contamination moved through the pathways we had hypothesized onto scrubs and skin.

Fluorescence experiments, however, carry limitations [13]. Fluorescence does not reflect sanitation measures and can be visually tracked by participants. Contamination experiments with phages resolve both failings. The viruses are visually undetectable and can be inactivated via alcohol-based sanitation but pose no appreciable risk [4, 14]. With the original doffing protocol, all participants had at least one contamination with at least 1800 virions present – an amount far greater than the minimum infective dose of many HCIDs (Supplementary Table 2).

We next offered an assortment of interventions. Modifications were made not only to reduce contamination by contact, but also to make doffing easier. Reducing discomfort for HCPs may reduce deviations from a protocol, reducing contamination. The result of these changes manifested in the phage experiment with the updated protocol. In the second phage experiment, we did not recover a viable population of phage on any of the nine HCPs. Through PCR, we found phage DNA in several locations, indicating that the phages were inactivated by

the use of alcohol-based sanitizer during the doffing. Phages that may have contaminated several locations were routed through pathways that included successful hand sanitation. Moreover, updates to the protocol eliminated intermediate contamination locations present in the original doffing protocol, which would have re-contaminated the HCP at later doffing steps. These results were observed despite deviations from the protocol by the HCPs during their doffings. The protocol, built to include redundancies and reduce events of contamination, allowed for small deviations without self-contamination.

This study does contain limitations. The original contamination was deliberately placed according to the fluorescent test with the intention of revealing contamination pathways. Thus, we cannot wholly capture contamination that would occur in a clinical setting – instead, we show how specific contamination can be tracked and eliminated through specific procedures. Further studies are needed to capture how contamination may move throughout a clinical environment, on PPE and otherwise. We further note that phages are only proxies. Using HCIDs for studies such as this is not ethical, but accordingly, we are closely approximating how they would function in a clinical setting through phages.

With the initial PPE ensemble and doffing protocol, contamination occurred that would have endangered the individual HCP and the community at large had it occurred with a dangerous pathogen. Through modifications of both protocol and

equipment, the doffing protocol was successfully improved from initially incurring dangerous amounts of contamination to eliminating viable contaminants in all cases. These tests did not pose a great financial burden. Excluding PPE costs, each trial cost less than $45 USD, and our interventions were modest. Based on our results, validation of other healthcare PPE protocols by quantitative methods such as those we employed here is both logistically feasible and informative. No hospital procedure is designed for failure, but with empirical validation, those procedures can ensure they provide necessary protection.

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Potential Conflicts of Interest

The authors have no potential conflicts of interest to declare.

Data Availability

All data generated for this manuscript are available in the manuscript or its supplementary material.

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TABLES

Table 1: Events of concern noted during annotation of the PPE doffing movie.

Event Description	Step Number
When removing the outer gloves, the sleeves of the coverall may come into contact with the front surface of the PAPR hood.	17
Removing the heavy-loading PAPR filter creates a high risk of aerosolization.	19
The tie at the neck of the PAPR is not fully covered by the apron and could potentially contaminate the gloves when it is broken.	21
Incidental contact to the inside of the PAPR hood may occur when reaching in to unzip the coverall.	27
When the coverall is being pulled down the PAPR hood is free to move about and may contact skin of participant.	28
When marching in place to remove coverall, incidental contact with the PAPR hood occurs.	30

When marching in place, the PAPR hood is free to move around, potentially generating aerosols.	30
When the coverall is removed from the legs, one is instructed to “keep your hands together.” Forearms came in contact with the front of the PAPR hood that is not covered by the apron (henceforth referred to as the PAPR hood Critical Triangle).	31
When disposing of the coverall, there is a risk of incidentally interacting with patient-facing surfaces should care not be taken when picking the coverall off the ground.	32*
When removing the PAPR hood, the back of the hood is pulled forward from the back of the head to cover the face shield. The corner of the PAPR hood can fold out so that the PAPR hood Critical Triangle is exposed and the HCP removing the PAPR has no way of seeing this.	37
When the PAPR hood is flipped forward, the back of the PAPR can contact the front of one’s scrubs.	38
While reaching back and grabbing the hood, there is a large amount of contact between bare forearms, scrubs, and the PAPR hood Critical Triangle.	37

When locating the edges of the visor, incidental contact with the face may occur.	38
General Procedural Notes	
The alcohol sanitation is only being performed on gloves and not the forearms or wrists.	

444 *This step in the movie deviated from the written protocol.

445

446 **Table 2: Concerns noted while reviewing movie of four HCPs doffing.**

Event Description	Step Number
When removing the heavy-loading filter, people tend to not be conscious of where it is and have a tendency to either swing it around, aerosolizing particles, or touch it to their PAPR hood.	19
During the stomping to remove the coverall, the PAPR hood moves around substantially, often coming into contact with the scrubs and in the forearms.	30

One HCP misinterpreted how they should hold their hands in front of their body when removing the coverall and put their clasped hands against the front of the PAPR hood.	31
Clasping the hands in front of the body when removing the coverall often results in the bare forearms interacting with the front and/or Critical Triangle* of the PAPR.	31
Any manipulation above the head post coverall removal puts the HCP's forearms in contact with the PAPR hood Critical Triangle.	37-39
The PAPR hood repeatedly bunches up or flips over near the shoulders.	
On short HCPs, the front of the PAPR hood folds in on itself easily.	
On particularly tall HCPs, the apron does not cover nearly as much of the coverall and PAPR as it does on shorter individuals.	

447 *Critical Triangle = The side of the PAPR hood, coveralls, and arm, which may be
448 exposed and facilitate contamination

449

450

451 **Table 3: Updates to the protocol.**

452

Protocol Amendments			
Old Protocol	**New Protocol**	**Revision**	**Comment**
No inner shoe liner	Calf-high shoe liner over shoes and pant legs	Add inner shoe cover	Makes doffing coverall easier
Regular length inner gloves	Extended cuff inner gloves	Change length of inner gloves	Reduces risk of exposed skin at wrist
Outer gloves donned before PAPR	Outer gloves donned last	Move step for donning outer gloves	Outer gloves are worn over sleeve of gown
Apron	Gown	Replaced apron with gown	Improved coverage of PAPR hood at shoulders and Critical Triangle

Heavy-loading filter removed after apron	Heavy-loading filter removed first	Move step for heavy-loading filter removal	Removes higher-contaminated items earlier in doffing protocol
No gown	Gown sleeves freed from outer gloves	Insert step to pull gown sleeve out of the outer glove cuff	Facilitates gown removal

FIGURE LEGENDS

Figure 1: Fluorescent visualization of areas and actions of concern during doffing. Experimental results of doffing with fluorescent markers present for specific actions of concern or highlighting areas of concern, as found during review of the doffing protocol movie. **Left (L):** Before; shows areas that may be of concern. **Right (R):** After; shows potential concerns by transfer of fluorescence. **(A)** The original PPE ensemble in natural light, both with the apron and with the apron removed. **(B)** Patient facing surfaces of the PPE not covered

by the apron which could become contaminated. **(C)** Critical Triangle area of the PAPR. **(D)** Interaction of the coverall sleaves with the inside of the PAPR hood. **(E)** Transfer of contaminate from the PAPR hood to the forearm that can occur during doffing. **(F)** Aerosolization of fine powder trapped on the heavy-loading filter.

Figure 2: Phage recovery after doffing PPE. Experimental results of doffing protocols performed by four HCPs with three bacteriophages initially inoculated on the PAPR Critical Triangle, coverall cuffs, and the back of the PAPR hood. Numbers inside each square represents the number of PFU/mL recovered from that location.

Figure 3: Bacteriophage recovery after doffing PPE with the altered protocols. Experimental results of the altered doffing protocols performed by nine HCPs with three bacteriophages initially inoculated on the PAPR Critical Triangle, coverall cuffs, and the back of the PAPR hood. An X denotes that the phage DNA from the origin location was found at that sampled location at the end of doffing via PCR. To test for viable phages below the limit of detection ($1x10^2$ PFU/mL) samples were incubated with a susceptible bacteria host and no viable phages were recovered from any HCP.

MOVIE LEGENDS

Movie 1: Original Training Video. The basis for Table 1. Slight deviations occur between this video and Supplementary Table 3.

Movie 2: Original Doffing Protocol. A demonstration of the original doffing protocol. The steps performed are detailed in Supplementary Table 3.

Movie 3: Updated Doffing Protocol. A demonstration of the updated doffing protocol. The steps performed are detailed in Supplementary Table 3.