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Phi 6 recovery from inoculated fingerpads based on elution buffer and methodology

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ABSTRACT

Phi 6 (Φ6) bacteriophage is a proposed surrogate to study pathogenic enveloped viruses including SARS-CoV-2—the causative agent of COVID-19—based on structural similarities, BSL-1 status, and ease of use. To determine the role of virus-contaminated hands in disease transmission, an enhanced understanding of buffer and method performance for Φ6 recovery needs to be determined. Four buffer types and three methodologies were investigated for the recovery of Φ6 from human fingerpads over a 30 min duration. Phosphate buffered saline (PBS), PBS + 0.1 % Tween, 0.1 M glycine + 3% beef extract, and viral transport medium were evaluated as buffers for recovery of Φ6 via a dish, modified glove juice, and vigorous swabbing method. Φ6 concentrations on fingerpads were determined at 0-, 5-, 10-, and 30-min post-inoculation. While there were observed differences in virus recovery across buffer and method types depending on the time point, log PFU recovery based on buffer type or methodology was not significantly different at any time point (P > 0.05). The results presented in this study will allow for future work on Φ6 persistence, transfer between hands and surfaces, and efficacy of hand hygiene methods to be performed using a well-characterized and validated recovery method.

1. Introduction

The transmission of enveloped viruses, which includes severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—the causative agent of COVID-19—has important implications for assessing risks in future outbreaks due to enveloped viruses. Direct and indirect contact transmission routes impact risk assessments. A key factor in virus contact transmission is the transfer rate between hands and surfaces, which is driven in part by virus survival on human hands. The stability of SARS-CoV-2 on human skin is unknown due to the hazardous limitations of viral exposure to humans, and thus, this is not an ethical approach in research (Hirose et al., 2020). SARS-CoV-2 must be studied within BSL-3 facilities, which is cost-prohibitive, inaccessible to many researchers, and is a major limitation to the number of studies that can be conducted on these types of highly transmissible and virulent pathogens (Gallandat and Lantagne, 2017; Aquino de Carvalho et al., 2017). Therefore, oftentimes surrogate viruses with a lower biosafety level status are used in place of the actual pathogen.

Φ6 (Φ6) is a lipid-enveloped bacteriophage and a member of the Cystoviridae family. It is a segmented double-stranded RNA bacteriophage and approximately 75 nm in diameter (Gonzalez et al., 1977). Φ6 infects Pseudomonas syringae pathovar phaseolicola as a host, which in addition to Φ6, has BSL-1 status (Prussin et al., 2018; Turgeon et al., 2014; Vidaver et al., 1973). Φ6 has emerged as a leading surrogate to study SARS-CoV-2 based on structural similarities of the phospholipid envelope, relatively short analysis time (24 h), cost-effective assays, and BSL-1 status (of both Φ6 and its host) that enables experiments to be performed without specialized facilities (Adcock et al., 2009; Aquino de Carvalho et al., 2017; Barros et al., 2021).

The importance of indirect contact transmission via fomites contaminated with respiratory viruses and subsequent risk of infection continues to be debated (Goldman, 2020). Weber and Stilianakis (2020) highlighted the limited available data on the inactivation of respiratory viruses on human skin, which greatly impacts quantifying transfer rates. To determine the utility of Φ6 as a surrogate to assess indirect contact transmission, the recovery and persistence of Φ6 on human hands need to be characterized. While many studies have been performed to evaluate the persistence of viruses on surfaces (Castano et al., 2021; Kampf et al., 2020; Otter et al., 2016; Stephens et al., 2019; Vasilikova et al., 2010; Whitworth et al., 2020; Wood et al., 2020) as well as the transfer between hands and surfaces (Ansari et al., 1988; Bean et al., 1982; Castano et al., 2021; Julian et al., 2010; Lopez et al., 2014; Mbithi et al.,

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2. Materials and methods

2.1. Φ6 production and inoculum preparation

Φ6 bacteriophage (HER102) and *Pseudomonas syringae* pathovar *phaseolicola* (Pph) (HER1102) were procured from Dr. Sylvain Moineau at Université Laval in Québec, Canada. Medium used for Φ6 propagation and Pph growth was Lysogeny Broth (10 g NaCl, 10 g tryptone, 5 g yeast extract/L ultrapure water, pH adjusted to 7.5 with HCl) (LC broth). A frozen stock of Pph was streaked on LC agar and incubated at 25 °C for 48 h to obtain an isolated colony. One day prior to trials, an isolated colony was added to 15 mL of LC broth and incubated at 25 °C, 100 rpm for 24 h, which resulted in a host concentration of approximately 7.2 log CFU/mL. To produce Φ6 stock, the Pph bacterial host (200 μL) was added to 5 mL of LC soft agar (0.7 % agar) followed by 100 μL of undiluted Φ6 (approximately 10 log plaque forming units (PFU)/mL). The prepared soft agar tubes with the host and virus were poured onto LC agar plates (1.5 % agar) via the double agar overlay assay (DAL) (Kroplinski et al., 2009). Once dried, plates were inverted and incubated at 25 °C for 24 h. For LC plates with a lacy-webbed appearance due to phage lysis of the Pph host, Φ6 was harvested from the LC soft agar layer with a 25 cm cell scraper (VWR, Radnor, PA), and contents were placed in a 50 mL centrifuge tube. Four mL of LC broth was then added to LC agar plates, swirled for 5 s, and transferred to the 50 mL centrifuge tube containing the scraped soft agar. Tubes were vortexed for 15 s to disassociate Φ6 from lysed bacterial cells and agar, and subsequently centrifuged for 10 min at 3000 x g, 4 °C. Following centrifugation, supernatant was passed through a 0.45 μm sterile polycethesulfone syringe filter (Whatman, Buckinghamshire, United Kingdom), and Φ6 stock (approximately 10 log PFU/mL) was stored at 4 °C until use.

Prior to fingerpad inoculation, a Φ6 inoculum of approximately 9 log PFU/mL was prepared by diluting the stock in LC broth. The same Φ6 inoculum was used throughout all experiments and maintained at 4 °C when not in use. Prior to each experiment, the concentration of the Φ6 inoculum was determined by performing ten-fold serial dilutions of the inoculum in sterile 1 × phosphate buffered saline pH = 7.4 (PBS), and Pph host (200 μL) and Φ6 dilutions (100 μL) were added to LC soft agar and plated in duplicate via the DAL as previously described.

2.2. Fingerpad preparation and decontamination

Fingerpads were prepared by washing hands with liquid hand soap (Equate, Wal-Mart Stores, Inc., Bentonville, AR) for 30 s, rinsing thoroughly, and air-drying completely. Fingers were soaked in 70 % ethanol for 30 s and air-dried completely. Each fingerpad was demarcated with a sterile 2 mL plastic centrifuge tube (9 mm diameter) by pressing the tube opening firmly on the fingerpad for 15 s (Ansari et al., 1988). Following virus inoculation and recovery, fingerpads were wiped with 70 % ethanol soaked Kimwipes (Kimtech Science, Roswell, GA), and hands were washed for 30 s with hand soap. This procedure was performed before each fingerpad inoculation event, with a maximum of two trials being performed per day (two hand preparation procedures). A single volunteer was used for the entirety of the study except for two trials in which they were unavailable, and a separate volunteer participated.

2.3. Fingerpad inoculation and time to recovery

Demarcated areas on index, middle, ring, and pinky fingerpads were inoculated with 10 μL of Φ6 at approximately 7-log PFU. After a 5 min wait period, Φ6 was recovered at 0, 5, 10, and 30 min, which was performed with three separate methods and four separate buffer combinations. Following recovery, the eluent was diluted in sterile 1 × PBS, and 250 μL of Pph host and 500 μL of sample were suspended in LC soft agar, poured onto LC agar plates in duplicate and incubated at 25 °C for 24 h. Undiluted eluent was plated for each method-buffer combination at a limit of detection ranging from 0.04 to 0.28 log PFU, which was influenced by the eluted buffer volume. Thumbpads were demarcated, inoculated with sterile 1 × PBS, and sampled with each method-buffer combination as negative controls.

2.4. Φ6 recovery based on buffer and method type

The four buffer types included 1 × PBS, 1 × PBS + 0.1 % Tween (WVR), 0.1 M glycine buffer + 3% beef extract (Hardy Diagnostics, Santa Maria, CA), and viral transport medium (VTM), which consisted of Hanks Balanced Salt Solution (Quality Biology, Gaithersburg, MD) with 2% of heat-inactivated (56 °C ± 1 °C, 30 min) fetal bovine serum (HyClone, Logan, UT) without gentamicin or amphotericin B (CDC, 2020). Three separate recovery methods were evaluated: a modified glove juice method, a dish method, and a vigorous swabbing method. The modified glove juice method was performed based on the glove juice method ASTM 1174 – 13 (ASTM, 2013) and was modified by lowering eluent volume and bag size for virus recovery from individual fingerpads. For the modified glove juice method, inoculated fingerpads were placed in a 2 × 3” poly bag (Edvision, Shenzhen, China) containing 2 mL of buffer, the demarcated fingerpad area was massaged by a researcher for 1 min, and the eluent was transferred to a sterile 2 mL tube for analysis. The dish method (based on ASTM E1838 – 17) was performed by rubbing the demarcated fingerpad area into a single well of a 6-well plate (Greiner Bio-One, Monroe, NC) containing 2 mL of one of the four buffers for 1 min with moderate pressure, after which fingerpads were scraped across the edge of the well to capture excess liquid (ASTM, 2017). The vigorous swabbing method was performed by swabbing the inoculated demarcated fingerpad area with a pre-wetted (1.5 mL buffer) PUR-Blue Sampler, dry, small polyurethane foam swab (World Bioproducts LLC, Woodinville, WA) in a sweeping, rotating motion for 10 s (Julian et al., 2010).

2.5. Statistical analysis

Each buffer and method combination was performed in technical duplicates with two experimental trials. Plaque forming unit values were log transformed prior to statistical analysis. Log PFU loss was calculated at each time point as the difference in the inoculum log PFU and the recovered log PFU for each experiment. Virus recovery efficiencies were calculated by dividing the recovered log PFU by the inoculated log PFU. A Welch’s two-sample t-test was performed to test for a significant difference in virus recovery efficiency based on hand preparation procedure (first versus second wash) as well as based on hand (left versus right) used. Two-way analysis of variance was
performed to compare virus recovery efficiencies for each buffer and method at each time point ($P = 0.05$). Mean values were compared with Tukey’s honest significant difference test ($P = 0.05$). All statistical analyses were performed in R version 3.6.2 (http://www.R-project.org).

3. Results

3.1. $\Phi 6$ log PFU recovery based on hand and hand preparation procedures

No significant difference in log PFU recovery was observed between trials performed on left- versus right-handed fingerpads at any time point ($P > 0.05$). Similarly, no significant differences were observed based on whether trials were performed after one versus two hand preparation procedures (30 s hand wash with antimicrobial soap, air dry, 30 s soak in ethanol, air dry) ($P > 0.05$). Although not significantly different ($P > 0.05$), slightly lower virus recovery was observed among all trials performed after one hand preparation procedure versus two. For example, when comparing the first versus the second hand preparation, 83 and 87 % virus recovery were observed after 0 min; 51 and 55 % virus recovery was observed after 5 min; 32 and 38 % virus recovery was observed after 10 min; and 17 and 23 % virus recovery was observed after 30 min, respectively.

3.2. Inoculated and recovered $\Phi 6$ log PFU for all buffer and method types

Among method types, the greatest eluent volume recovered was from the dish method, followed by the bag and vigorous swabbing method at 95, 90, and 73 %, respectively. The mean ± standard deviation $\Phi 6$ log PFU inoculated on fingerpads for all trials was 6.88 ± 0.35. No significant difference in log PFU recovery was observed between buffer or method types at any time point ($P > 0.05$). When evaluating recovery efficiencies among all data regardless of buffer or method type, the mean ± standard deviation of virus recovery at 0, 5, 10, and 30 min was 84 ± 13 %, 53 ± 16 %, 34 ± 13 %, and 19 ± 10 %, respectively (Fig. 1). The cost of materials associated with each method at the time of purchase are listed in Table 1.

4. Discussion

4.1. Virus recovery from human fingerpads over time

Virus recovery from human skin can be influenced by several factors such as virus type, inoculum level, droplet size, skin characteristics (pH, microbiota, sebum content), temperature, and relative humidity (Byrd et al., 2018; Carducci et al., 2002; Pitol et al., 2018; Prussin et al., 2018; Thomas et al., 2014). This research was performed to determine the most optimal elution buffer to recover $\Phi 6$ from fingerpads via a vigorous swabbing method, dish method, and modified glove juice method. This study will guide future research on the efficacy of hand hygiene products with immediate and residual antiviral effects for $\Phi 6$ inactivation as well as research related to virus transfer from surfaces to hands.

A virus loss of approximately 1 log PFU at time 0 min (5 min wait) and 3 log PFU at time 5 min (10 min total) was observed in this study across all buffer and method types. Grayson et al. (2009) observed a 3–4 log loss of influenza A virus—a common enveloped respiratory virus—on hands after a 2 min drying time, which was similar to a previous study investigating influenza A virus on hands and fingers (Schürmann and Eggers, 1983). While not all studies report the baseline $\Phi 6$ recovery levels from hands or fingerpads following inoculation (Wolfe et al., 2017), Casanova and Weaver (2015) observed a 2–4 log loss immediately following inoculation of $\Phi 6$ on whole hands in which virus was recovered by the glove juice method with mechanical rubbing of volunteer hands. Instantaneous inactivation of $\Phi 6$ on fingerpads has also been observed when recovered byimmersing and agitating fingers in a conical tube containing neutralizing solution for 10 min (Woolwine and Gerberding, 1995).

It should be noted that fingerpads in the current study remained ‘wet’ for 15–20 min post inoculation. Following inoculation of fingerpads for analysis of handwash and handrub agents for ASTM International Standard E1838 – 17, fingerpads are allowed to dry for 15–30 min prior to treatment. In this study, the log PFU loss across all buffers and methods was $4.57 \pm 0.87$ at 10 min sampling (15 min total) and $5.58 \pm 0.71$ at 30 min (35 min total). Thus, results reported here suggest a

![Fig. 1. Virus recovery efficiency over time on fingerpad (min) for the modified glove juice (Bag), dish, and vigorous swabbing (Vig. Swabbing) methods with glycine buffer (closed circle), PBS (open triangle), PBS + 0.1 % Tween (closed circle), and viral transport medium (closed square). Mean virus recovery efficiency for each method and buffer is represented by the red line.](image-url)
detrimental influence of drying on Φ6 survival, which is consistent with previous studies on inoculum drying and virus inactivation (Brady et al., 1990; Firquet et al., 2015).

4.2. Virus recovery between trials performed on same day

Virus recovery during the second trial for same-day analyses was slightly higher in comparison to the first trial at each time point in this study. Similar observations were noted by Casanova and Weaver (2015) across 6 trials evaluating Φ6 loss from whole hands. These authors observed a significant difference between trials 1 and 2-6 but not between trials 2 through 6. The presence of oil deposits and transient microbial populations on human skin may influence virus recovery after a single wash and may be removed or reduced on subsequent hand-/fingerpad decontamination steps prior to further trials (Kownatzki, 2003). However, no significant differences were observed between left- and right-hand log virus loss in this study similar to a previous finding (Casanova and Weaver, 2015).

4.3. Comparison of methods used for virus recovery

As indicated previously, various methods for virus recovery from hands and/or fingerpads have been reported. Previous fingerpad testing assessments have incorporated a plastic vial inversion method, in which the vial containing the buffer is placed over the inoculated demarcated area of the fingerpad and inverted repeatedly for virus recovery (Ansari et al., 1988; Mbithi et al., 1992; Rotter et al., 2009; Sattar and Ansari, 2002). Unfortunately, there is limited amount of data on the dish method investigated in this study by incorporating sampling bags that are proportional to a right-hand log virus loss in this study similar to a previous finding (Casanova and Weaver, 2015).

The glove juice method, which incorporates whole hand sampling from inoculated hands, has been utilized previously by Casanova and Weaver (2015) as well as Wolfe et al. (2017) for Φ6 recovery. A potential limitation of whole hand sampling is the larger surface area that may limit efficient recovery of the virus; thus, this method was modified in this study by incorporating sampling bags that are proportional to a finger. While no significant differences in virus recovery efficiency were observed based on method type in this study, the dish method (ASTM, 2017) resulted in higher virus recovery in comparison to the modified glove juice method and vigorous swabbing methods at 0- and 30-min recovery times and was second behind the modified glove juice method at 5- and 10-min sampling time points. In addition to having the highest eluent recovery (95%), the dish method may be preferable based on the ease of use when compared to the modified glove juice and vigorous swabbing method. While based on personal experience and opinion, we attribute our preference for the dish method in part to how easily the eluent can be recovered from the wells following fingerpad rubbing. This was not the case for the vigorous swabbing method in which the eluent was vortexed following fingerpad swabbing to disassociate the virus from the swab and suspend the virus in the buffer. This resulted in an eluent with numerous bubbles that may have limited virus recovery, which did not occur with the dish or modified glove juice method. Additionally, the lower eluent recovery volume (73%) during vigorous swabbing can also be attributed to the hold volume of the swab material (Jones and Gibson, 2020).

Lower eluent recovery can be problematic when quantifying the recovered virus levels from fingerpads. In addition to foaming and hold volume issues associated with the vigorous swabbing method, liquid loss and dispersal during the 10 s of vigorous swabbing action on fingerpads were observed. Efforts were made to completely pass over the demarcated area, while simultaneously rotating the swab for complete swab coverage, and this may have contributed to sample volume loss. While greater eluent volumes were recovered from the modified glove juice method in comparison to the vigorous swabbing method, recovering all of the eluent at the bottom corner of the bag was problematic, making it less optimal in comparison to the dish method.

4.4. Influence of buffer type on virus recovery

Virus recovery from various matrices (biotic/abiotic surfaces, foods, wastewater) often hinges on the type of elution buffer (Brehm-Stecher et al., 2009; Turnage and Gibson, 2017; Ye et al., 2016). Beef extract exhibits a high ionic strength and protein content to desorb viruses from surfaces and has been investigated as a means to increase virus recovery (Carducci et al., 2002; Pitol et al., 2017; Verani et al., 2014). However, while beef extract did not impact virus recovery, higher recovery efficiency was observed with viral transport medium containing fetal bovine serum, which has shown to increase virus recovery in previous studies (Watanabe et al., 1988; Winona et al., 2001). Previously, the influence of buffer type on Φ6 recovery from the whole hand indicated slightly higher recovery when using beef extract in comparison to PBS, Tween (0.1 and 0.01 %), tryptic soy broth, and NaCl (Casanova and Weaver, 2015). Casanova and Weaver (2015) reported a range of 2.4–3.8 log loss across all buffer types when sampling hands by the glove juice method immediately following inoculation of Φ6 onto hands at approximately 8 log PFU/mL in a total 4.5 mL. In this study, 0 min samples were collected after a 5 min wait period following inoculation of demarcated fingerpads. A lower log loss was observed across all method and buffer types in this study at only 1.10 ± 0.92 log PFU loss after a 5 min wait (time 0 min) but was similar at the 5 min sampling time point (10 min total) at 3.29 ± 1.13 log PFU loss. The greater virus recovery at time 0 in this study compared with Casanova and Weaver (2015) is likely due to the wet inoculum still present at 0 min, after which an approximately 2 log PFU reduction was observed between the 0- and 5-min sampling time points.

5. Conclusions

The survival time of Φ6 on human hands has implications for future research focused on appropriate surrogates for highly pathogenic enveloped viruses. Optimizing virus recovery from human fingerpads is a critical step prior to characterizing the risk of direct and indirect contact transmission associated with human skin. Future studies to determine the most appropriate elution buffer and methodology for virus recovery from fingerpads should consider eluent recovery volume, recovery efficiencies, material costs, and ease of use for both the volunteer and researcher. Overall, Φ6 can be easily implemented to study virus persistence, transfer, and inactivation in relation to SARS-CoV-2 and other enveloped viruses relevant to public health (Castano et al., 2021).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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