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Project Page Link: <https://review.jove.com/account/file-uploader?src=21617533>

Title: Evaluating the Effect of Coated Filters and Pre-Filters on the Infectivity and Concentration of Aerosolized Phi6 Virus

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 15

Number of Shots: 40

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Note: The statements have been edited for length and clarity

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Svetlana Sofieva-Rios:** We investigate the effect of pre-filters and antiviral coatings in reducing airborne virus infectivity and concentrations in indoor settings.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Svetlana Sofieva-Rios:** Current approaches miss realistic airflow dynamics; our protocol recreates aerosol conditions and measures both infective viruses and total viral load.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Svetlana Sofieva-Rios:** This protocol allows researchers to quantify viral inactivation, filtration efficiency, and differences between infectious particles and total viral load.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Svetlana Sofieva-Rios:** A key challenge is very short virus-surface contact time during airflow, which limits the effectiveness of antiviral coatings.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Svetlana Sofieva-Rios:** Future studies can explore combining coatings with UV or testing performance in real-world ventilation systems and with diverse viruses.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.6. **Nina Atanasova, Senior Researcher, University of Helsinki:** (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

How does the video format of your article help you to share your work with the broader scientific community?

- 1.7. **Nina Atanasova, Senior Researcher, University of Helsinki:** (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Efficiency of Anti-viral Surface Coatings Against Phi6 Bacteriophage

Demonstrator: Rasmus Malmgren

2.1. To begin, dilute the purified Phi6 (*Fie-six*) bacteriophage stock suspension in 20 millimolar potassium phosphate buffer containing 1 millimolar magnesium chloride, adjusted to pH (*P-H*) 7.2 [1-TXT]. Assemble all the filter, pre-filter and anti-viral coating materials within the biosafety cabinet to perform the surface tests [2]. Assess the anti-viral activity of all coating materials based on copper and-or-or silver active components embedded in proprietary matrices, namely A6 (*A-six*), B4 (*B-four*), VS (*V-S*) and VS-B (*V-S-B*) on flat test pieces [3].

2.1.1. WIDE: Talent sitting at the biosafety cabinet and diluting the bacteriophage stock for surface testing experiments. **TXT: Viral titer = $3 \times 10^{11} - 1.2 \times 10^{12}$ PFU/mL**

Videographer: Please obtain CU shot as well for this shot.

2.1.2. Talent assembling all filter, pre-filter and anti-viral coatings materials.

2.1.3. LAB MEDIA: Table 1. *Video Editor: Please show only the column labelled "Coatings".*

2.2. Next, pipette twenty microliters of diluted Phi6 bacteriophage stock solution onto coated test pieces to perform the surface viability assay [1]. Spread the sample evenly over a 2cmx 2cm (*two-by-two square centimeters*) area using a sterile glass triangle [2]. Incubate the samples at twenty-two degrees Celsius up to four time points including one minute, five minutes, one hour or three hours [3].

2.2.1. Talent pipetting 20 μ L of Phi6 stock solution onto a coated test piece.

2.2.2. Talent spreading the solution using a glass triangle across the surface.

2.2.3. Talent placing samples aside for incubation within the biosafety cabinet.

2.3. Add one eighty microliters of fresh LB (*L-B*) broth to the surface to recover the viruses after each timepoint [1]. Wash the surface by repeated pipetting ten to twenty times to collect the sample [2]. Use a plastic Petri dish as control surface [3].

2.3.1. Talent adding LB broth onto the test surface.

2.3.2. Talent pipetting up and down to wash and collect the sample.

- 2.3.3. Close-up of a plastic Petri dish labelled as control.
- 2.4. Quantify antiviral efficacy of the surface coatings using plaque assay to measure the number of infectious viral particles [1] and quantitative reverse transcriptase polymerase chain reaction or qRT-PCR (*Q-R-T-P-C-R*) to determine viral genome copy numbers [2].
 - 2.4.1. Close up shot of plaqued plates to show the viral titer.
 - 2.4.2. Representative shot of qPCR software interface showing amplification curves and data output. **Author's NOTE:** I've attached an alternative image (*Näyttökuva 2026-05-29 122145.png*) to use for 2.4.2 (qPCR software), if the video of the computer screen doesn't work properly.

3. Virus Aerosolization and Filter or Pre-Filter Testing

Demonstrator: Svetlana Sofieva-Rios

- 3.1. Dilute the purified and concentrated Phi6 bacteriophage suspension as demonstrated previously to obtain a viral concentration in the range of 2.5×10^{10} to 4.08×10^{11} plaque-forming units per milliliter [1]. Next, assemble the experimental aerosol chamber using a cylindrical glass body with a matching lid [2]. Insert the internal metal scaffold into the chamber [3] and secure insulating gaskets to ensure an airtight seal [4].
 - 3.1.1. WIDE: Talent diluting the concentrated bacteriophage suspension.
 - 3.1.2. Talent placing the cylindrical glass body and the matching lid.
 - 3.1.3. Talent inserting the internal metal scaffold into the chamber.
 - 3.1.4. Talent positioning insulating gaskets and sealing the chamber.
- 3.2. Use a nebulizer in combination with an air pump to generate a stable and reproducible input of viral aerosols [1]. Titrate the virus solution used in the nebulizer before and after each experiment to monitor stability [2]. Set the aerosol flow rate to eight liters per minute within the chamber [3].
 - 3.2.1. Talent connecting the nebulizer and the air pump to the chamber.
 - 3.2.2. Talent collecting the virus samples for analysis before and after nebulization through the chamber.
 - 3.2.3. Close-up of the setup showing the flow rate of 8 liters per minute.
- 3.3. To reduce humidity during prolonged measurements, direct approximately three-quarters of the aerosol flow to the exhaust through a High-Efficiency Particulate Air, or HEPA (*HEP-uh*) filter [1]. Allow the remaining one-quarter of the flow, corresponding to two liters per minute, to enter the chamber [2]. Use a metal funnel to direct the aerosol flow toward the filter or pre-filter surface within the chamber [3].
 - 3.3.1. Talent adjusting tubing to route airflow toward the HEPA filter.

- 3.3.2. Close-up of airflow entering the chamber at reduced rate.
- 3.3.3. Talent positioning a metal funnel to direct aerosol flow onto the filter surface.
- 3.4. Dehumidify the outgoing airflow using a desiccator to protect the HEPA filter and flow meter from excessive moisture [1]. Using the external pump, create negative pressure within the system and further support airflow to ensure efficient exhaust through the outlet port [2].
 - 3.4.1. Talent connecting the chamber outlet to a desiccator.
 - 3.4.2. Talent operating an external pump connected to the system
- 3.5. Next, evaluate the antiviral properties of uncoated or VS-B coated polyester and bulpren pre-filters, alongside double-layer glass fiber materials [1], within the aerosol chamber [2].
 - 3.5.1. Talent placing the various uncoated and coated pre-filter and filter materials in the biosafety cabinet near the chamber for evaluation.
 - 3.5.2. TEXT ON A PLAIN BACKGROUND
Prefilters:
 - 1) FMR-5 (Polyester prefilter)
 - 2) S-11 (Polyester prefilter)
 - 3) BP-6 (Bulpren prefilter)

All pre-filters were evaluated in both uncoated and VS-B coated forms.

Filters:

 - 1) GF-2 (Double-layer glass fiber HEPA-type filter)
- 3.6. To evaluate the anti-viral effect by impaction sampling, position a Petri dish containing 20 millimolar HEPES (HEEP-eez) buffer at pH 7.2 directly beneath the testing pre-filter to maximize particle deposition [1]. Alternatively, to test the anti-viral effect of GF-2 (G-F-two) filters, set up a triple-nozzle, all-glass swirling impinger connected to an external pump for aerosol collection [2].
 - 3.6.1. Talent placing a Petri dish beneath the pre-filter for impaction sampling.
 - 3.6.2. Talent removing the Petri dish from setup, replaces the tested pre-filter with GF-2 filter and attaches impinger to the exhaust line filter.
- 3.7. Maintain airflow through the testing pre-filter or filter at two liters per minute [1]. Increase total air influx to ten liters per minute using an additional valve after the chamber outlet [2]. Capture the aerosols in five milliliters of 20 millimolar HEPES buffer and replenish evaporated buffer every thirty minutes [3].
 - 3.7.1. Close-up of airflow control showing 2 liters per minute setting.
 - 3.7.2. Talent adjusting a valve to increase airflow to ten liters per minute.
 - 3.7.3. Talent adding buffer to maintain liquid volume.

- 3.8. For triplicate sampling, perform control runs without pre-filters or filters for fifteen minutes before and after each experiment [1]. After the pre-experiment control run, insert the filter or pre-filter into the chamber and operate the system for three hours [2]. Collect viral aerosols by recovering one milliliter of suspension from Petri dishes containing twenty milliliters of buffer every thirty minutes [3]. Remove the filter and conduct a post experiment control run [4].
 - 3.8.1. Talent performing a control run in the chamber without a filter.
 - 3.8.2. Talent inserting the desired pre-filter or filter and operating the system.
 - 3.8.3. Talent collecting 1 mL liquid sample from the Petri dish using a pipette.
 - 3.8.4. Talent removing the filter and repeating the control run.
- 3.9. Collect samples every thirty minutes during the experiment to obtain eight qRT-PCR and titration samples each per experiment [1]. In impinger experiments, collect titration samples only once at the end of the three- hour run [2].
 - 3.9.1. Talent placing the 8 labelled samples collected at timed intervals in a stand for downstream qPCR analysis.
 - 3.9.2. Talent collecting a single sample at the end of the impinger experiment.
- 3.10. Use eight hundred microliters of the collected sample for qRT-PCR analysis [1] and the remaining two hundred microliters for virus titration [2].
 - 3.10.1. Talent transferring 800 microliters into a labeled tube for qRT-PCR analysis.
 - 3.10.2. Talent using the remaining 200 microliters for virus titration analysis.
- 3.11. Snap freeze qRT-PCR samples in liquid nitrogen and store them at minus eighty degrees Celsius [1]. Process virus titration samples immediately using plaque assay [2].
 - 3.11.1. Talent placing sample tubes into liquid nitrogen.
 - 3.11.2. Representative shot of talent performing the plaque assay.

Results

5. Results

- 5.1. The surface viability assays demonstrated that all tested coatings successfully inactivated the virus, with significant reductions observed within the first five minutes [1]. The copper-based surface coating, B4 (*B-four*) achieved the fastest reduction, decreasing infectious counts by three orders of magnitude within five minutes [2] and reaching complete inactivation with detection limit below hundred plaque forming units per milliliter by fifteen minutes [3].
 - 5.1.1. LAB MEDIA: Figure 2A and 2B. *Video Editor: Show both Figure 2A and 2B. In figure 2B, emphasize the bars labelled as VS, VS-B, A6 and B4 sequentially.*
 - 5.1.2. LAB MEDIA: Figure 2B: *Video Editor: Please highlight the difference between the two bars labelled as B4. NOTE: If the authors provide an updated figure with Y-axis label, use that (from the published manuscript on the webpage).*
 - 5.1.3. LAB MEDIA: Figure 2A: *Video Editor: Please highlight the red dot within the graph and the column between 0 to 20 on the X-axis simultaneously.*
- 5.2. A6 (*A-six*) coating achieved total virus inactivation within twenty-five minutes [1] and VS-B within sixty minutes [2]. VS (*V-S*) coating exhibited the slowest inactivation rate among the tested materials [3].
 - 5.2.1. LAB MEDIA: Figure 2A: *Video Editor: Please highlight the grey dots within the graph and the column between 0 to 20 on the X-axis simultaneously.*
 - 5.2.2. LAB MEDIA: Figure 2A: *Video Editor: Please highlight the green dot between the X-axis lines labelled 20 and 40.*
 - 5.2.3. LAB MEDIA: Figure 2A: *Video Editor: Please highlight the yellow dot on the X-axis line labelled 180.*
- 5.3. The studied prefilters BP-6 (*B-P-six*), S-11 (*S-eleven*), and FMR-5 (*F-M-R-five*) were equally effective in lowering the number of infectious viruses passing through them by one-point five to two orders of magnitude compared to the no-filter control [1]. The GF-2 (*G-F-two*) filters demonstrated superior performance, lowering the viral load by at least six orders of magnitude and no infectious Phi6 virus plaques were detected within the detection limit [2]. The VS-B coating did not significantly affect infectious virus filtration efficacy compared to uncoated pre-filters [3].
 - 5.3.1. LAB MEDIA: Figure 3A and 3B: *Video Editor: In figure 3A, please emphasize the brown, green and blue solid lines sequentially. In figure 3B, please emphasize the brown, green and blue solid fill bars sequentially.*

- 5.3.2. LAB MEDIA: Figure 3A: *Video Editor: Please emphasize the grey dotted line along with the corresponding Y-axis value.*
- 5.3.3. LAB MEDIA: Figure 3A: *Video Editor: Please emphasize the brown solid and dotted lines together, followed by green solid and dotted lines, then blue solid and dotted lines.*
- 5.4. The qRT-PCR results showed that pre-filters lowered the viral genome copy numbers by up to two orders of magnitude [1]. The GF-2 filter showed a decrease of up to four orders of magnitude compared to the no-filter control [2]. While the differences between pre-filters and control remained insignificant during the first thirty minutes [3], the mechanical filtering efficiency increased from the sixty-minute time point [4]. Additionally, the application of the VS-B coating had no measurable effect on the number of viral genomes passing through the filtration media [5].
- 5.4.1. LAB MEDIA: Figure 4A: *Video Editor: Please emphasize the brown solid and dotted lines together, followed by green solid and dotted lines, then blue solid and dotted lines in comparison with black solid line.*
- 5.4.2. LAB MEDIA: Figure 4A: *Video Editor: Please emphasize the Y-axis values corresponding to the grey line and the black line along with the lines simultaneously.*
- 5.4.3. LAB MEDIA: Figure 4A: *Video Editor: Please emphasize all lines except the grey line corresponding to X-axis value 30.*
- 5.4.4. LAB MEDIA: Figure 4A: *Video Editor: Please emphasize all lines except the grey line beyond X-axis value 60.*
- 5.4.5. LAB MEDIA: Figure 4B: *Video Editor: Please emphasize the brown solid and patterned bars together, followed by green solid and patterned bars, then blue solid and patterned bars.*
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1. Bacteriophage
Pronunciation link: <https://www.merriam-webster.com/dictionary/bacteriophage>
IPA: /bæk'tɪrɪəˌfeɪdʒ/
Phonetic Spelling: bak·teer·ee·uh·fayj
 2. Phi6
Pronunciation link: No confirmed link found
IPA: /faɪ sɪks/
Phonetic Spelling: fie·six
 3. Potassium
Pronunciation link: <https://www.merriam-webster.com/dictionary/potassium>

- IPA: /pəˈtæsiəm/
Phonetic Spelling: puh·tas·ee·uhm
4. Phosphate
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /ˈfɑːs, fert/
Phonetic Spelling: fos·fayt
5. Magnesium
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnesium>
IPA: /mægˈniːziəm/
Phonetic Spelling: mag·nee·zee·uhm
6. Biosafety
Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>
IPA: /ˌbaɪoʊˈseɪfti/
Phonetic Spelling: bye·oh·sayf·tee
7. Antiviral
Pronunciation link: <https://www.merriam-webster.com/dictionary/antiviral>
IPA: /ˌæntiˈvaɪrəl/, /ˌæntaɪˈvaɪrəl/
Phonetic Spelling: an·tee·vye·ruhl; an·tie·vye·ruhl
8. Viability
Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>
IPA: /ˌvaɪəˈbɪləti/
Phonetic Spelling: vye·uh·bil·uh·tee
9. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /ˈɪŋkjəˌbeɪt/
Phonetic Spelling: ing·kyuh·bayt
10. Plaque Assay
Pronunciation link: <https://www.merriam-webster.com/dictionary/plaque>
IPA: /plæk/; /ˈæseɪ/
Phonetic Spelling: plak; a·say
11. Quantitative
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantitative>
IPA: /ˈkwɑːntəˌteɪtɪv/
Phonetic Spelling: kwon·tuh·tay·tiv
12. Transcriptase
Pronunciation link: <https://www.howtopronounce.com/transcriptase>
IPA: /trænˈskɹɪptəs/
Phonetic Spelling: tran·skrip·tays
13. Polymerase
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>
IPA: /pəˈlɪməˌreɪs/
Phonetic Spelling: puh·lim·uh·rays
14. Aerosolization
Pronunciation link: <https://www.howtopronounce.com/aerosolization>

- IPA: /ˌerəˌsɑːləˈzeɪʃən/
Phonetic Spelling: air·uh·sol·uh·zay·shuhn
15. Nebulizer
Pronunciation link: <https://www.merriam-webster.com/dictionary/nebulizer>
IPA: /ˈnebjələˌzər/
Phonetic Spelling: neb·yuh·luh·zer
16. Particulate
Pronunciation link: <https://www.merriam-webster.com/dictionary/particulate>
IPA: /pərˈtɪkjələt/
Phonetic Spelling: per·tik·yuh·luht
17. HEPA
Pronunciation link: <https://www.merriam-webster.com/dictionary/HEPA>
IPA: /ˈhepə/
Phonetic Spelling: hep·uh
18. Desiccator
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccator>
IPA: /ˈdesəˌkeɪtər/
Phonetic Spelling: dess·uh·kay·ter
19. Polyester
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyester>
IPA: /ˌpɑːliˈestər/
Phonetic Spelling: pol·ee·es·ter
20. Bulpren
Pronunciation link: No confirmed link found
IPA: /ˈbʊlpren/
Phonetic Spelling: bull·pren
21. Impaction
Pronunciation link: <https://www.merriam-webster.com/dictionary/impaction>
IPA: /ɪmˈpækʃən/
Phonetic Spelling: im·pak·shuhn
22. HEPES
Pronunciation link: <https://www.howtopronounce.com/hepes>
IPA: /ˈhiːpiːz/
Phonetic Spelling: heep·eez
23. Impinger
Pronunciation link: <https://www.howtopronounce.com/impinger>
IPA: /ɪmˈpɪndʒər/
Phonetic Spelling: im·pin·jer
24. Titration
Pronunciation link: <https://www.merriam-webster.com/dictionary/titration>
IPA: /taɪˈtreɪʃən/
Phonetic Spelling: tie·tray·shuhn
25. Filtration
Pronunciation link: <https://www.merriam-webster.com/dictionary/filtration>

IPA: /fɪlˈtreɪʃən/

Phonetic Spelling: fil·tray·shuhn

26. Inactivation

Pronunciation link: <https://www.merriam-webster.com/dictionary/inactivation>

IPA: /ɪnˌæktəˈveɪʃən/

Phonetic Spelling: in·ak·tuh·vay·shuhn

27. Infectious

Pronunciation link: <https://www.merriam-webster.com/dictionary/infectious>

IPA: /ɪnˈfektʃəs/

Phonetic Spelling: in·fek·shuhs

28. Genome

Pronunciation link: <https://www.merriam-webster.com/dictionary/genome>

IPA: /ˈdʒiːˌnoʊm/

Phonetic Spelling: jee·nohm

29. Mechanical

Pronunciation link: <https://www.merriam-webster.com/dictionary/mechanical>

IPA: /məˈkænɪkəl/

Phonetic Spelling: muh·kan·ih·kuhl

30. Efficacy

Pronunciation link: <https://www.merriam-webster.com/dictionary/efficacy>

IPA: /ˈefəkəsi/

Phonetic Spelling: ef·uh·kuh·see