# Persistence of Viral Nucleic Acids in Wastewater in Presence of Surfactants

Allie (Minh Anh) Nguyen*[1], Audrey (Li-Wen) Wang [1,2], Staci R. Kane [3], Kara L. Nelson *[1]

1. Department of Civil and Environmental Engineering, University of California, Berkeley, CA, USA
2. Department of Bioscience, Rice University, Houston, TX, USA
3. Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA

*allienguyen@berkeley.edu, karanelson@berkeley.edu

Berkeley Civil & Environmental Engineering

## Motivation

- Wastewater surveillance monitors circulating viral pathogens by quantifying their nucleic acid signals in sewage.
- Surfactants are commonly discharged into wastewater and can degrade nucleic acid signals, potentially biasing surveillance measurements.
- **Study objective: Quantify how four surfactant types affect the decay of eight virus targets over 72 hours in influent wastewater.**

## Key Takeaways

- For enveloped viruses, signal decay was higher in wastewater than PBS.
- Higher signal decay in the presence of added surfactants occurred almost exclusively in enveloped viruses.
- Ionic surfactants (anionic SDS, cationic DDAC) were more likely to cause higher decay; nonionic Tween 80 had minimal effect.
- Extraviral (free) nucleic acids degraded rapidly in wastewater, with the concentration decreasing by 2 to 4 $\log_{10}$ within 90 minutes.

## Results and Findings

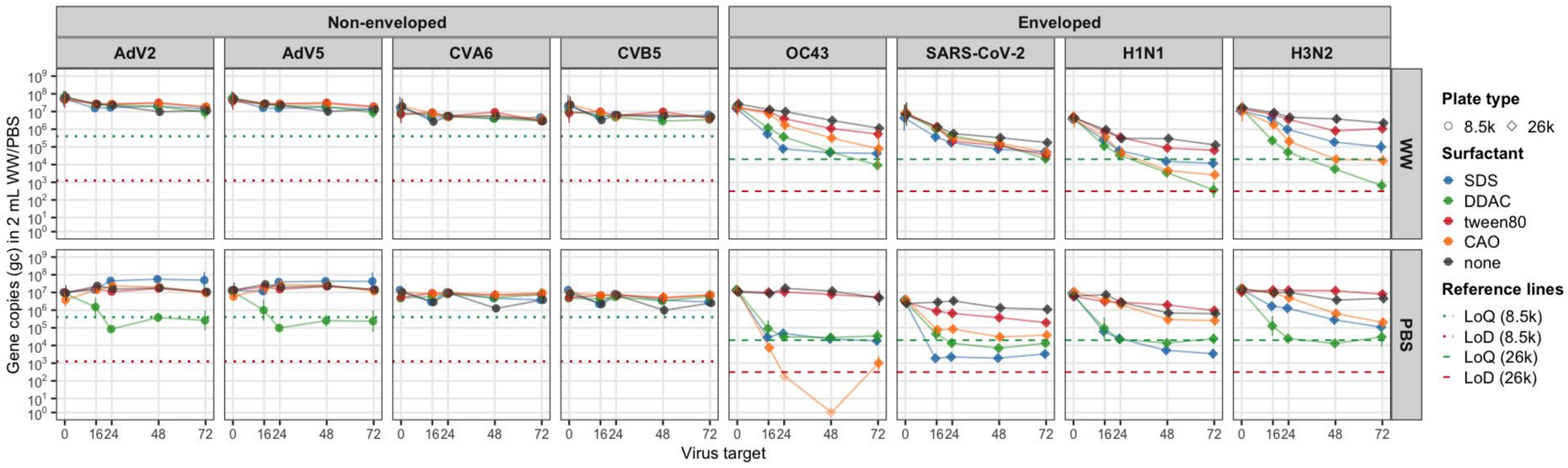



**Figure 1: Decay of eight viral nucleic acid targets in wastewater and PBS over 72 hours, with and without added surfactants.**

- Nonenveloped viruses showed little to no signal decay in wastewater across all surfactant conditions, except DDAC (AdV2 and AdV5 decay in PBS).
- Enveloped viruses decayed by 2 to 4 $\log_{10}$ over 72 hours in the presence of surfactants.
- For enveloped viruses with no surfactants spiked in, signal decay was more pronounced in wastewater than PBS.

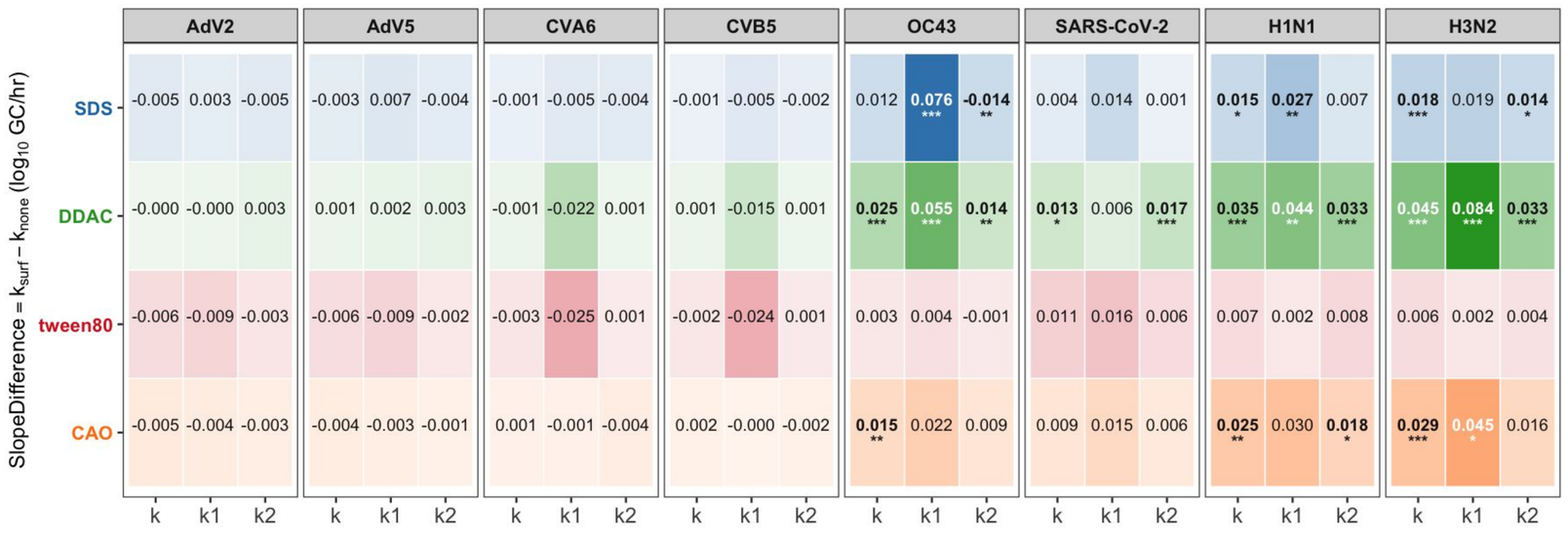



**Figure 2: Difference between decay rates with and without surfactant ($k_{surf}$ - $k_{none}$; wastewater only). k = monophasic; $k_1$ and $k_2$ = early (0-24 h) and late (24-72 h) biphasic rates. Stars: * p < 0.05, ** p < 0.01, *** p < 0.001.**

- Significant surfactant effects were observed for enveloped viruses.
- SDS (anionic) and DDAC (cationic) most consistently caused higher decay rates.
- Analysis using both biphasic ($k_1$, $k_2$) and monophasic decay models provided more holistic understanding.
- Tween 80 (nonionic) had the weakest effects, consistent with surfactant charge driving envelope disruption.

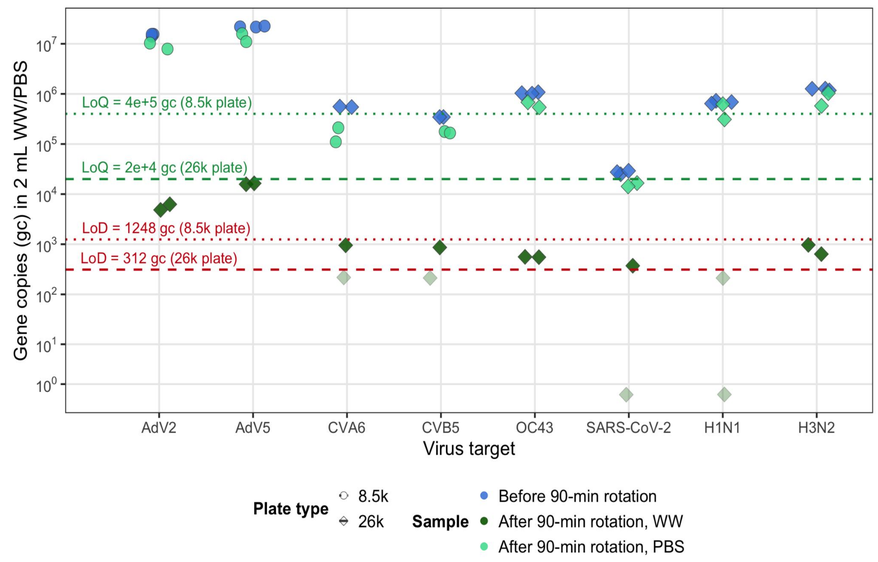
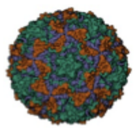
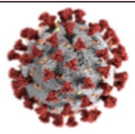
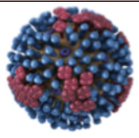


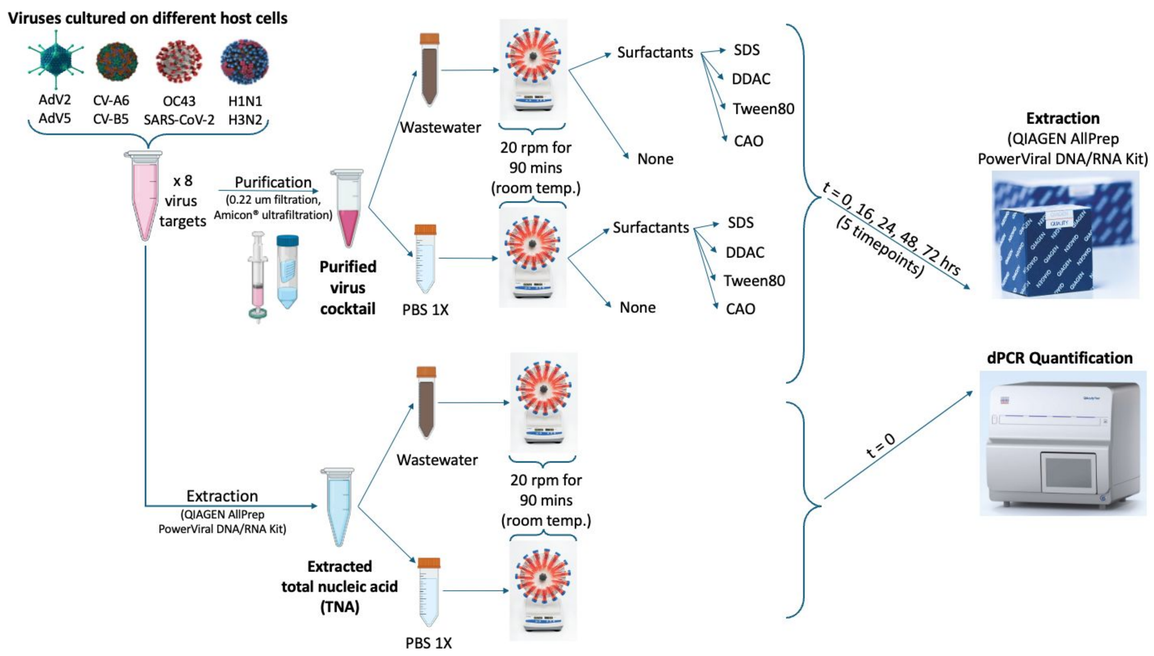

**Figure 3. Concentration of extraviral (free) nucleic acid spiked into wastewater before and after 90 min incubation.**

- Extraviral nucleic acid degraded rapidly, decreasing 2 to 4 $\log_{10}$ within 90 min.
- This finding confirms that the t=0 signal in the decay experiment derived predominantly from intact virus particles rather than free nucleic acids.

## Methods

| Adenoviruses | Enteroviruses | Coronaviruses | Influenza A |
|---|---|---|---|
|  |  |  |  |
| Adenovirus 2, Adenovirus 5 (AdV2, AdV5)<br>• Non-enveloped<br>• dsDNA | Coxsackievirus A6 and B5 (CV-A6, CV-B5)<br>• Non-enveloped<br>• ssRNA | SARS-CoV-2 and OC43<br>• Enveloped<br>• ssRNA | H1N1 and H3N2<br>• Enveloped<br>• ssRNA |

| Surfactant Name | Abbreviation | Surfactant Group |
|---|---|---|
| Sodium dodecyl sulfate | SDS | Anionic (-) |
| Didecyl dimethyl ammonium chloride | DDAC | Cationic (+) |
| Polysorbate 80 | Tween 80 | Nonionic |
| Cocamidopropylamine oxide | CAO | Zwitterionic (±) |




## Acknowledgements

Funding for the Pandemic Readiness and Equity through Virus Epidemiology of Wastewater (PREViEW) project was provided by the UC National Laboratory Fees Research Program (UCOP Lab Fees CRT Award L22CR4507). We thank David Williams, Adam Kern, and Melody Tseng at the East Bay Municipal Utility District (EBMUD) for their assistance with wastewater sample collection, and Jordan Boeck at UC Davis for help with virus culturing.

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- Surfactants are commonly discharged into wastewater and can degrade nucleic acid signals, potentially biasing surveillance measurements.
- **Study objective: Quantify how four surfactant types affect the decay of eight virus targets over 72 hours in influent wastewater.**

## Key Takeaways

- Surfactant signal decay occurred almost exclusively in enveloped viruses (OC43, SARS-CoV-2, H1N1, H3N2), not in nonenveloped viruses (AdV2, AdV5, CVA6, CVB5).
- Ionic surfactants (anionic SDS, cationic DDAC) produced the strongest and most consistent decay; nonionic Tween 80 had minimal effect.
- Free nucleic acids degraded rapidly in wastewater, with the concentration decreasing by 2 to 4 log-10 within 90 minutes.

## Results and Findings

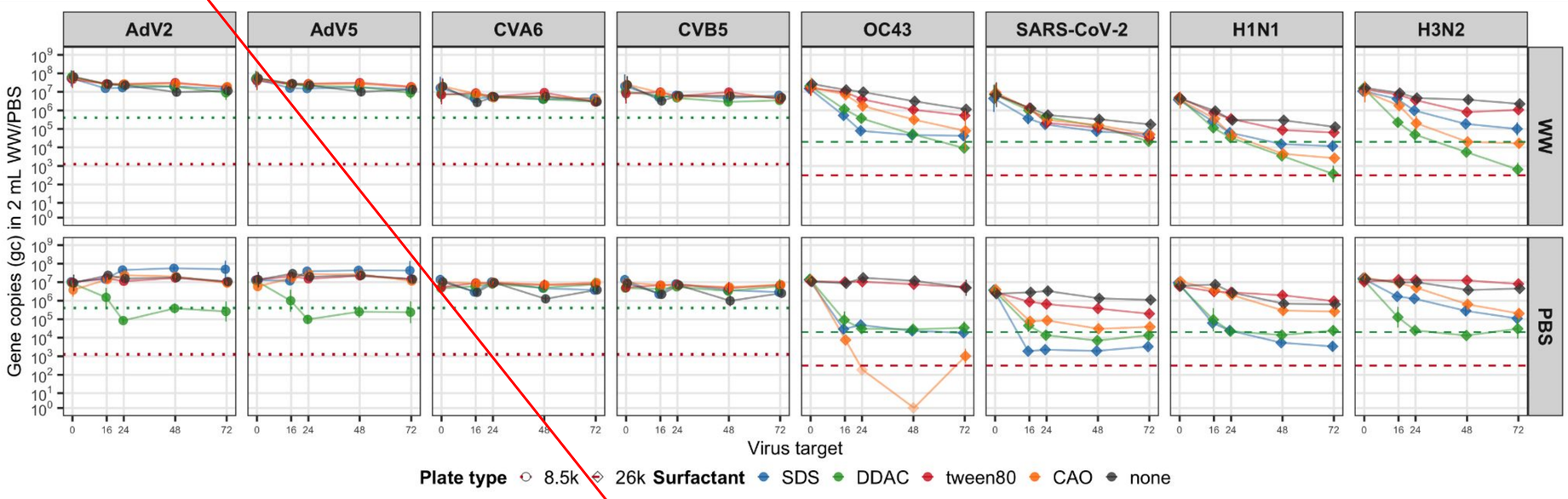



**Figure 1: Decay of eight viral nucleic acid targets in wastewater and PBS over 72 hours, with and without added surfactants.**

- Nonenveloped viruses showed little to no decay in wastewater across all surfactant conditions, except DDAC, which degraded AdV2 and AdV5 in PBS.
- Enveloped viruses decayed by 2 to 4 log10 over 72 hours in the presence of surfactants.
- Susceptibility tracked envelope status, not genome type: the non-enveloped RNA viruses CVA6 and CVB5 behaved like the nonenveloped DNA viruses.

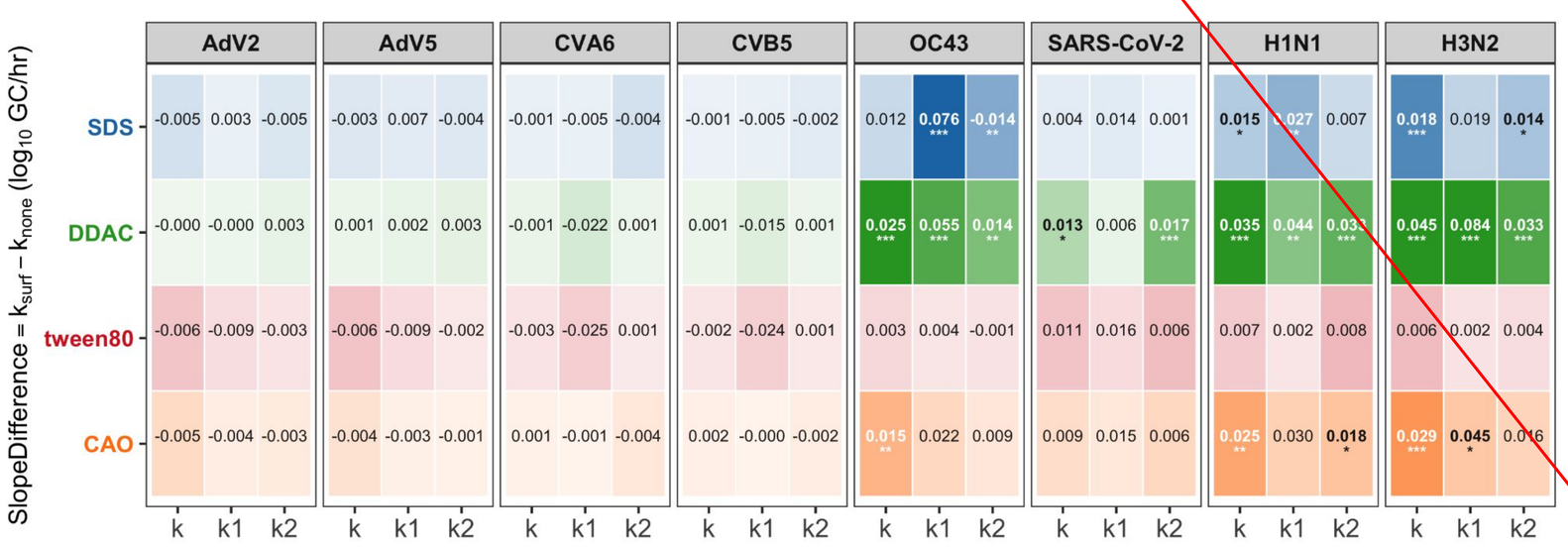



**Figure 2: Comparison of the decay rate of each surfactant vs no surfactant (wastewater only).**

k = monophasic; k1 and k2 = early (0-24h) and late (24-72h) biphasic rates.

Stars: * p<0.05, ** p<0.01, *** p<0.001.

- Significant surfactant effects were concentrated in enveloped viruses.
- SDS (anionic) and DDAC (cationic) most consistently accelerated decay.
- Phase-resolved analysis (k1, k2) detected effects that the single-rate model missed.
- Tween 80 (nonionic) had the weakest effects, consistent with surfactant charge driving envelope disruption.

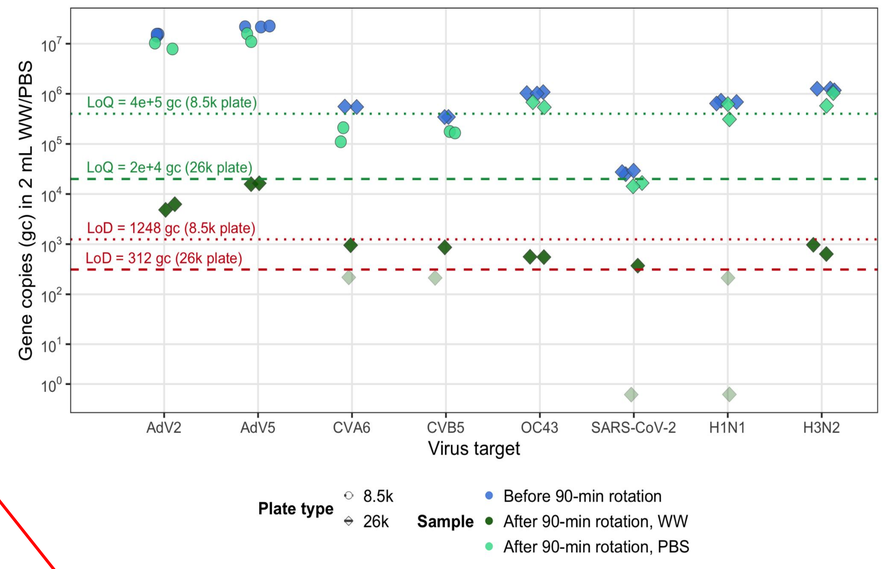



**Figure 3. Concentration of extraviral nucleic acid spiked into wastewater before and after 90 minutes of rotation**

- Extraviral nucleic acid degraded rapidly, dropping 2 to 4 log10 within 90 minutes.
- This confirms that the t=0 signal in the decay experiment derived predominantly from intact virus particles rather than free nucleic acid.

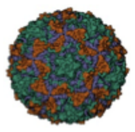
## Methods

### Adenoviruses

Adenovirus 2, Adenovirus 5 (AdV2, AdV5)

- Non-enveloped
- dsDNA

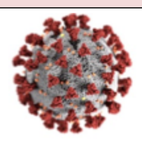
### Enteroviruses



Coxsackievirus A6 and B5 (CV-A6, CV-B5)

- Non-enveloped
- ssRNA

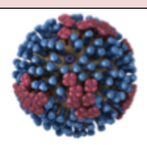
### Coronaviruses



SARS-CoV-2 and OC43

- Enveloped
- ssRNA

### Influenza A



H1N1 and H3N2

- Enveloped
- ssRNA

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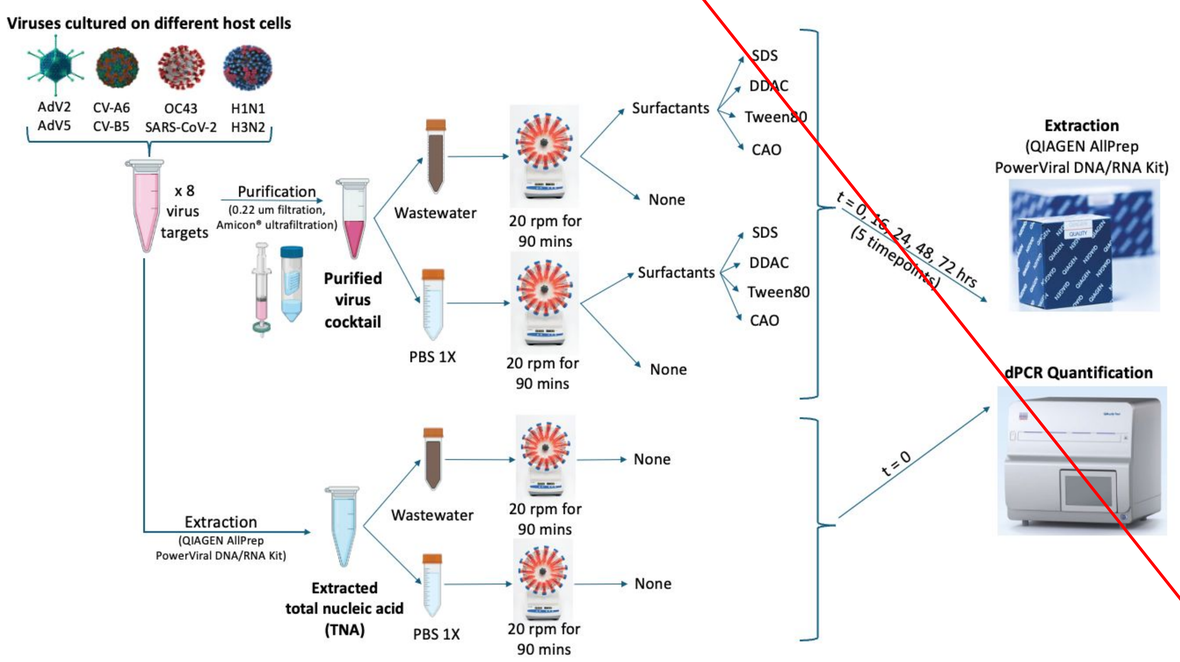


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