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Step-by-step protocol: IAV titration by Dynamic Light Scattering

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We use this protocol and it's working

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Abstract

This protocol describes a standardized approach for quantifying Influenza A virus (IAV) titers using Dynamic Light Scattering (DLS). The method includes sample preparation, ensuring proper viral supernatant clarification to remove unwanted particulate material, followed by measurement optimization based on virus-specific parameters such as size, shape, and aggregation tendency. The protocol also details correlation with standard viral titration methods, enabling the establishment of a calibration curve for accurate viral quantification.

The expected results include reproducible DLS measurements, with a hydrodynamic diameter within 120 ± 30 nm, a polydispersity index (Pdl) typically between 20–25%, and a strong correlation between DLS intensity and plaque assay titers (PFU/mL). The troubleshooting section addresses common challenges, such as sample aggregation, contamination, and intensity fluctuations, providing strategies to enhance data reliability.

Materials

1. Laboratory equipment

- Dynamic Light Scattering (DLS) instrument (e.g., Litesizer 500, or equivalent)
- Refrigerated centrifuge capable of at least $6,000 \times g$ (e.g., Heraeus Megafuge 16R)
- Class II Biosafety Cabinet (if working with infectious virus samples)
- Pipettes (1–10 μ L, 20–200 μ L, 100–1,000 μ L)

2. Consumables

- Polystyrene disposable cuvettes (10 $\times$ 10 $\times$ 45 mm) (e.g., Anton Paar, 67754)
- Filtered pipette tips (10 μ L, 200 μ L, 1,000 μ L)
- 1.5 mL microcentrifuge tubes
- 15 mL and 50 mL conical tubes
- Sterile syringe filters (0.22 μ m, PVDF)

3. Reagents and solutions

- Influenza A virus (IAV) supernatants (infected and uninfected "mock" samples)
- Dulbecco's Modified Eagle Medium (DMEM) or RPMI, depending on viral propagation conditions, pH 7.2–7.4
- PBS (Phosphate-Buffered Saline), pH 7.4
- Fetal Bovine Serum (FBS) (as required)

4. Software and data analysis

- DLS instrument software (e.g., Kalliope, or equivalent)
- Graphing software (e.g., GraphPad Prism, Excel)

5. Biosafety and handling materials

- Nitrile or latex gloves
- Lab coat & safety goggles
- Face mask



Before start

During viral propagation assays, supernatants from an infected culture and an uninfected control (mock) were obtained and clarified as described on IAV supernatants clarification section on main manuscript. Clarification step is crucial as it removes suspended material of no interest that would increase light scattering, obscuring the effect of viral particles.

IAV supernatants clarification

- 1 The samples were centrifuged at $6,000 \times g$ at $4\text{ }^{\circ}\text{C}$ for 30 min using a Heraeus Megafuge 16R centrifuge (Thermo Scientific) with a fixed-angle rotor (radius: 8.3 cm, model: F15-6 $\times$ 100, Thermo Fisher). After centrifugation, the supernatants were carefully transferred to new sterile tubes without disturbing the pellet. Finally, the clarified supernatants were stored at $-80\text{ }^{\circ}\text{C}$ until further titration.

Samples preparation

- 2 Dispense a sufficient volume of sample into the cuvette [polystyrene disposable cuvette 10 $\times$ 10 $\times$ 45 mm (67754, Anton Paar)] to obtain reliable measurements. Refer to your instrument's user manual for specific volume recommendations, but typically, a volume between 700 and 100 μL is adequate. 
- 2.1 NOTE: Exercise caution when transferring the sample to avoid introducing particulate matter (such as dust) or generating microbubbles, which can compromise the accuracy of the reading. It is advisable to let the cuvette stand undisturbed for a minimum of 5 minutes prior to measurement.
- 2.2 NOTE: Tests were also performed with 500 μL capacity cuvettes and the readings were reproducible (results not shown).
- 2.3 PRECAUTION: If working with potentially infectious samples, or in laboratories without biosafety levels for virus containment, it is recommended to inactivate viral particles by a method that does not alter their structure, such as ionizing radiation. Protocols for this have been previously reported[1,2]. 

Establishment of sample-specific parameters

- 3 Precise measurement of samples, achieved by closely monitoring the technique's variables, is fundamental for ensuring reproducible and valid results. To accomplish this, it is essential to have a thorough understanding of the specific characteristics of the virus being quantified, such as possible sizes, shapes, tendency to aggregate or form multimers. This is explained in more depth in the related manuscript.
- 3.1 NOTE: Filamentous forms, which can extend several micrometers in length, introduce significant measurement challenges by scattering light anisotropically. This can lead to overestimation of hydrodynamic diameter and an increased polydispersity index. Additionally, aggregates may further deviate size distribution readings. To ensure reliable and reproducible DLS results in viral titration studies, careful sample preparation for filamentous forms and aggregates separation is recommended to validate particle morphology and improve data accuracy. 



- 4 In the case of IAV supernatants, measurements were conducted after 3 min of stabilization time, with a detector angle of 90°, a temperature of 25 °C, and an equilibration time of 30 s. Data was collected in triplicate, with each replicate consisting of 30 acquisitions of 10 s each. The analysis model was generally adjusted, and the cumulative model was specifically adjusted according to ISO 22412:2017[3]. The filter and focus settings were automated. Considering the enveloped nature of the virus, the material was defined as exosomes with a refractive index of 1.3700 and an absorption coefficient of 0.0100 m⁻¹, while the solvent was assigned a refractive index of 1.3304 and a viscosity of 0.0009259 Pa·s (these values will depend on the culture medium used and the percentage of FBS. In the work of Poon C., 2022[4] you can consult it for DMEM or RPMI at different conditions). Reproducibility of readings, a good fit in the correlation function curve (exponential decay), and a hydrodynamic diameter within the expected range of 120 ± 30 nm[5,6] were used as acceptance criteria for valid measurements. Additionally, a Pdl of 20 – 25 % is typically acceptable for viral samples, as some degree of heterogeneity is inherent to biological systems. However, values approaching 30 % or higher may indicate excessive aggregation or insufficient clarification, potentially compromising size estimation. Therefore, careful sample preparation and proper interpretation of Pdl values are essential to ensure accurate viral quantification by DLS.
- 4.1 **DISCLAIMER:** These conditions should be tested for each virus. We determined that for the Influenza A PR8 virus, good reproducibility was obtained.
- 4.2 **NOTE:** Before starting the readings, ensure that the calibration of your DLS equipment is up-to-date and suitable for the size range of the viral particles. If not, perform the process using a reference standard of known size according to the manual instructions.
- 4.3 **NOTE:** The absolute intensity should not exceed the recommended limit of 600 kcounts/s to ensure reliable and reproducible measurements[7,8]. If this threshold is surpassed, gradually dilute the sample using the appropriate solvent (e.g., DMEM without FBS) until the intensity falls within the acceptable range.
- 4.4 **RECOMMENDATION:** If you need to reuse your viral particles for other assays, taking advantage of the non-destructive nature of this technique, you should consider the working conditions of the samples, i.e., maintain the sterility of the materials used and keep the samples refrigerated until reading to allow for subsequent use.



Correlation of DLS measurements with standard viral titration methods

- 5 For each new virus tested, a calibration curve must be generated to accurately correlate viral titers obtained by DLS with those determined by a reference method. This curve is established by plotting the DLS light scattering intensity against viral titers measured using a gold standard technique (e.g., plaque assay for IAV) or a well-established alternative (e.g., TCID₅₀)[5]. Once validated, the calibration curve can be routinely applied in the laboratory within the defined concentration range, ensuring consistent and



reliable viral particle quantification. The supernatant from the uninfected cell culture (mock) served as a blank control. The established calibration curve should exhibit a linear relationship between the absolute intensity (kcounts/s) measured by DLS and the viral titer obtained by plaque assay (PFU/mL). The interval where the viral supernatant readings intersect with those of the mock should be excluded to ensure the accuracy of the correlation.

Measurement of unknown samples

- 6 Viral supernatants with unknown titers also underwent viral clarification. DLS measurements were performed under the same controlled conditions as those established for the standard curve. To determine the viral titer, the average absolute light intensity was calculated and interpolated onto the standard curve equation, using the formula $y = mx + b$, where y represents the absolute light intensity and x corresponds to the equivalent viral titer.

- 6.1 NOTE: If any reading falls above the standard curve, dilutions can be made with the same medium used for propagation culture, keeping FBS concentration (important to keep viscosity). Finally, adjust the viral titer of the measurement by multiplying by the dilution factor used.



Data validation, analysis and report

- 7 For each laboratory, it is necessary to validate the method by performing the standard curve as a positive control and the dilution of the uninfected supernatant (mock) as a negative control. Additionally, it is recommended to compare the results with more than one standard viral titration methods, if possible. Given the nature of the measurement, it is recommended that the viral titers determined by this technique be expressed as PFU/mL equivalents (eqPFU/mL) or TCID₅₀/mL equivalents (eqTCID₅₀/mL).

Troubleshooting

- 8 Accurate DLS measurements require careful sample preparation and handling to minimize artifacts caused by contaminants, aggregation, or improper dilution. This section provides guidance on common issues encountered during measurements, including unexpectedly large particle readings, abnormal intensity trace profiles, deviations in scattered light intensity, and excessive polydispersity. By following these troubleshooting steps, users can ensure reliable and reproducible results while maintaining sample integrity.
- 8.1 Readings show particles with micrometer diameters: This could be due to the presence of bubbles, dust particles, or other contaminants. It is highly recommended to use capped cells. If possible, prepare a new sample or allow the sample to settle in the cell

for 5–10 minutes to allow contaminants to precipitate and bubbles to escape. Try repeating the measurement. Another possibility is the aggregation of viral particles. If the viruses tend to aggregate, they should be dispersed by gradual dilution, ensuring that the concentration is reduced incrementally to prevent aggregation while maintaining detectable signal levels. Use the appropriate buffer or medium (e.g., DMEM with FBS) to preserve viral integrity during the process.

- 8.2 Intensity trace graphs show one or two very pronounced peaks: This indicates the presence of contaminants. If possible, change the sample or, if not, let it settle for about 10 min for the larger particles to precipitate. Another option is to centrifuge the sample (see details in the previous section) to remove contaminants. If this does not work, try extending the reading time for each run and increasing the equilibration time between readings.
- 8.3 Issues with absolute measurements of scattered light intensity: It is recommended to keep measurements between 20 and 600 kcounts/s[7]. If you obtain a higher reading, dilutions must be made. If the reading is below the lower limit, the sample is too dilute and the measurement must be repeated using a higher concentration. In our experience, making base 10 dilutions is a good strategy and is less time-consuming.
- 8.4 Readings show a population with a polydispersity greater than 30 %: This is likely due to the formation of small viral aggregates, very similar-sized contaminants, or the asymmetry of the viruses being measured. Try diluting the sample to reduce contaminants or disperse any possible aggregates. Consult specific literature on the expected symmetry of the viruses under study, considering their origin, to rule out this possibility.

Protocol references

1. Sadraei M, Zhang L, Aavani F, Biazar E, Jin D. Viral inactivation by light. *eLight*. 2022;2: 1–18. doi:10.1186/s43593-022-00029-9
2. Cuapa-González MA, Santos-López G, Orduña-Díaz A, Martínez-Gutiérrez H, Rojas-López M. Detection of Zika Virus by the Development of a Colloidal Gold Nanoparticle-Based Immunosensor. *Anal Lett*. 2024. doi:10.1080/00032719.2024.2313708
3. ISO. ISO 22412:2017 - Particle size analysis — Dynamic light scattering (DLS). In: Geneva, Switzerland [Internet]. 2016 [cited 8 Aug 2024]. Available: <https://www.iso.org/standard/65410.html>
4. Poon C. Measuring the density and viscosity of culture media for optimized computational fluid dynamics analysis of in vitro devices. *J Mech Behav Biomed Mater*. 2022;126. doi:10.1016/j.jmbbm.2021.105024
5. Eisfeld AJ, Neumann G, Kawaoka Y. Influenza A virus isolation, culture and identification. *Nat Protoc*. 2014;9: 2663–2681. doi:10.1038/nprot.2014.180
6. Driskell JD, Jones CA, Tompkins SM, Tripp RA. One-step assay for detecting influenza virus using dynamic light scattering and gold nanoparticles. *Analyst*. 2011;136: 3083–3090. doi:10.1039/c1an15303j
7. Bellmann C, Caspari A, Moitzi C, Babick F, Schäffler M, Luxbacher T, et al. Dynamic and Electrophoretic Light Scattering: Guidelines for particle-size analysis and zeta-potential determination. Anton Paar GmbH. 2019.
8. Farrell E, Brousseau J-L. Guide for DLS sample preparation. Brookhaven Instrum. 2014.

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