Al-Turath Medical Journal (ATMJ)
Open Access Journal
Open Access Journal
ISSN Print: 3080-1265
Online : 3080-1273

Reviewer Paper

Polyethylene Glycol 6000 Precipitation for low-titer virus detection: Optimization Strategies and Mitigation of Inhibitor Interference

Anmar Layth Talib, Jabbar S. Hassan
Medical Microbiology/ College of Medicine, Al-Nahrain University/ Baghdad, Iraq
Correspondence email | jabbarsalman30@yahoo.com
Received: 27/05/2026 | **Accepted**: 28/06/2026 | **Available online:** 30/06/2026 | **DOI:** https://doi.org/10.63964/atmj.2026.2.2.1

ABSTRACT
Polyethylene glycol (PEG 6000) precipitation has been identified as an efficient pre-analytical procedure for determining the concentration of viral fragments via clinical and natural specimens' collection, especially in low viral load conditions. The accuracy of viral load, especially by polymerase chain reaction, is necessary to diagnostic of different viral infections. PEG 6000 enhances the analytical sensitivity of post-processing molecular techniques, including polymerase chain reaction, by decreasing the volume of samples and increasing the density of virus particles. This method is particularly useful for wastewater – based epidemiological research as well as for detecting latent or persistent viral infection. Nevertheless, PEG-mediated precipitation processes can co-isolate inhibitor compounds which interact with amplification performance; it requires thoughtful optimization and confirmation, given the benefits. Consequently, PEG 6000 offers an economically feasible and flexible strategy for viral enhancement with significant consequences of improving both the accuracy and sensitivity associated with research as well as medical laboratory diagnostic applications. This review provides a comparative assessment of inhibitor mitigation strategies.

KEYWORDS: *Polyethylene glycol, PEG 6000, viral-mediated precipitation, wastewater*

Review Methodology
This narrative review was applied depend on some studies that conducted to identify the application of polyethylene glycol (PEG 6000) precipitation for viral concentration and the mitigation of PCR inhibitors. Electronic databases, including Google Scholar, PubMed, ScienceDirect, Scopus and Web of Science, were systematically searched for articles published between 1988 and 2025. The search strategy employed combinations of the following keywords and their related terms: polyethylene glycol 6000, PEG precipitation, virus concentration, viral load, PCR inhibitors, wastewater surveillance, viral recovery, molecular diagnostics, and RT-qPCR. Studies were considered qualifying for this review if they demonstrated the use of PEG 6000 for viral concentration, evaluated viral recovery performance, valued approach techniques for PCR inhibitor removal or mitigation, likened viral concentration methods, or provided appropriate methodological or clinical perception into molecular viral detection. Handiest articles published in English and to be had as full-text peer-reviewed publications have been blanketed. Publications restricted on bacterial or fungal applications, conference abstracts, duplicate publications, articles lacking enough experimental or methodological data, and reviews irrelevant to PEG-based viral concentration have been excluded. The regained literature was explored for relevance based on the title, abstract, and full-text review, and the best informative and scientifically robust studies were selected to offers a comprehensive assessment of PEG 6000-mediated virus precipitation, its applications, current challenges, and strategies for minimizing inhibitor interference in molecular diagnostic assays.

1-INTRODUCTION

Quantifying viral load is one of the cornerstones of advanced virology and has an impact on clinical assessment, treatment surveillance and infection control approaches. Despite improvements in molecular diagnostics, the detection of viruses in low viral burden samples is still problematic. Consequently, pre-analytical specimen processing procedures and specific viral concentration measurement methods are necessary (1,2). PEG 6000 is a type of hydrophilic polymer that is typically used to precipitate macromolecules like viruses or other proteins (3,4). The use of it within virology has generated rising interest given its ease of use, capacity for expansion and alignment with biological assays (5). PEG 6000 induces viral precipitation through the volume exclusion effect, whereby PEG molecules reduce the solvent volume available to viral particles, thereby decreasing their solubility and promoting aggregation.

Polymethyl glycol promotes the aggregation of viral particles formation in a solution of salt (usually Nacl), and both of these can be purified by using centrifugation (6,7). Wastewater consists of a variety of possible PCR

inhibitors from the surroundings (8). the number of viral particles can additionally give rise to concentration and simultaneous purification of atypical inhibitors, which could severely decrease PCR sensitivity (9). Research studies also indicate which probes and primers are rarely equally affected by different inhibitors, and can give rise to excessive and inadequate representation of some virus target groups (RNA and DNA) in a specimen (10). The reason for this represents a non -specific process, suitable for many different kinds of viruses, which include both enveloped or non- enveloped viruses as well as DNA or RNA viruses, although recovery effectiveness might differ.

1- Principle of PEG 6000-Mediated Virus Precipitation

Polyethylene glycol 6000 is a widely utilized polymeric compound in the field of molecular biology, used to purify viruses, which is an essential procedure for accurate measurement of virus concentration and different proteins. The basic principle of action of the PEG molecule fills the space and attaches to water, which reduces the volume of liquid solvent accessible to virus particles (4,11). The macromolecular crowding effect can leads to the aggregation and precipitation of the viruses which consequently this makes it possible to separate and concentrate the viruses from a larger volume of samples (12). The process of purifying the sample is essential to improve the sensitivity of the following up detection processes, such as PCR or RT-PCR, in particular when viruses are found at low concentrations (13,14). Figure (1).

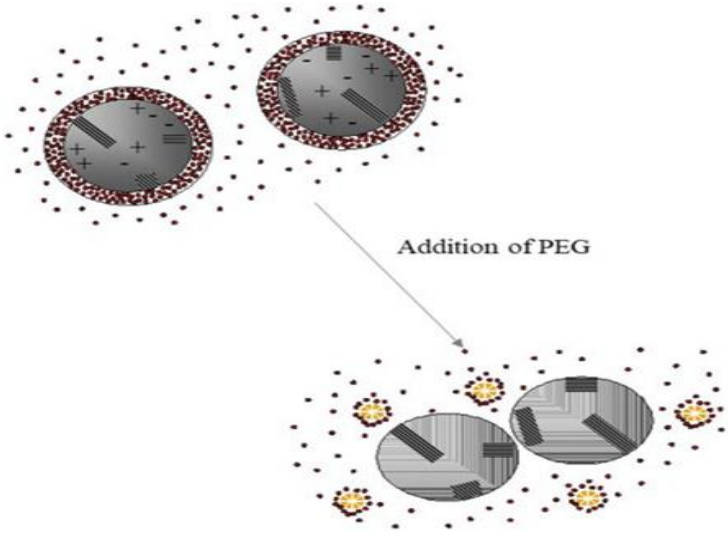

Figure 1. The volume exclusion mechanism by which PEG 6000 reduces the solvent volume available to viral particles, subsequently promoting aggregation and precipitation (17).

2- Polyethylene Glycol 6000 precipitation Methodology

The standard process is required by PEG600 (8-12%) concentration, and 2% (w/v) NaCl to incubate the sample at 4 °C for several hours to overnight, which was added to the samples, such as serum, plasma and other fluids, allowing for virus aggregate formation by volume exclusion (15). A high-speed centrifugation was performed to separate the particles of the virus. The supernatant was carefully decanted, and the resulting pellet was re-suspended in a smaller amount of buffer to improve nucleic acid extraction as well as to improve the sensitivity of viral load quantification of viral load detection by PCR (16).

3- Application of PEG 6000 in viral load:

a-Clinical Samples: In the context of the clinical virology field, PEG 6000 was commonly utilized for concentrating viral particles coming from serum, plasma, stool, wastewater and various other biological samples in order to optimize nucleic acid extraction (18). Thus, greatly enhances the sensitivity of analysis about viral load tests for clinically important pathogens like hepatitis C, hepatitis B, cytomegalovirus, HIV, norovirus, rotavirus and COVID-19 infections and is evaluated by using quantitative PCR and reverse transcription PCR methods (19,20).

b-Environmental and wastewater monitoring: PEG 6000 is a common method used in wastewater -based epidemiology for precipitation and detection of different viruses, particularly during epidemics. This improves viral recovery from big volume specimens and allows for quick detection of widespread. These techniques it acts like a molecular sponge, absorbing water and changing the soluble state of viral particles, so that they may be readily formed and extracted as usual (6,21,22).

4- Effect on detection sensitivity at the molecular levels:

Precipitation with PEG 6000 significantly improves the analytical sensitivity of the molecular diagnosis test by focusing viral particles and preserving viral nucleic acid from degradation before extraction (23).This level of concentration procedure increases the quantity of target DNA or RNA obtainable for amplifying it, leading to reduced cycle threshold (CT) value in different real time PCR assays, enhanced limitation of detection and increased reliable detection of samples with low levels of viruses. The use of PEG 6000 also reduces the stochastic variation associated with small template quantities and enhances the consistency of the testing, which reduces the risk of false negative findings, especially in samples with viral levels near the limit of the detection threshold (12). PEG-based concentration measurement methods are thus particularly useful in clinical diagnosis, epidemiological monitoring and basic research, in which high sensitivity is necessary for accurate identification and quantification of viral infections (24).

5- Advantages of PEG 6000 precipitation methods:

The method is actually simple and does not need advanced equipment like ultracentrifuges, which makes it readily available during routine laboratory testing and research institutions with limited funds. PEG 6000 is affordable and stable in chemical form, simple to prepare, and thus offers relatively low running expenses and high capacity for expansion for processing of small sized human specimens or large-volume sample sizes such as wastewater or environmental monitoring specimens (25). Moreover, the precipitation procedure is simple and maintains the structural strength of the virion and the stability of viral nucleic acid, allowing for efficient downstream extraction and accurate detection by Real time PCR and next generation sequencing (26). The procedure is highly repeatable, amenable to automation and easily standardized among labs, which promotes research comparison and consistency. Taken together, these features render PEG 6000 precipitation a convenient and dependable concentration method to increase viral recovery and improve the sensitivity of molecular assays in healthcare and public health contexts (12).

6. Comparison of strategies used to mitigate PCR inhibitors

Polyethylene Glycol 6000 precipitation is a widely adopted and cost-effective method for concentrating viruses, it frequently co-precipitates substances that inhibit downstream molecular assays, particularly RT-qPCR. Common inhibitors include humic acids, fulvic acids, polysaccharides, proteins, bile salts, heavy metals, and residual PEG. In order to increase nucleic acid purity and analytical sensitivity, a number of post-concentration purification techniques have been developed. Among these, DAX-8 resin treatment which is non -Ionic polymeric adsorbent has reported boss removal of humic substances from environmental samples, from other hands magnetic bead-based purification and silica column extraction meaningfully eliminate residual contaminants while preserving viral nucleic acids. Ultrafiltration provides an effective alternative for concentrating viruses while reducing low-molecular-weight inhibitors, although membrane fouling may limit its efficiency. Sample dilution remains the simplest approach but inevitably decreases viral nucleic acid concentration, potentially reducing assay sensitivity. Accordingly, the choice of an inhibitor mitigation strategy should be based on the sample matrix, target virus, and downstream molecular application to achieve the optimal trade-off between viral recovery and inhibitor removal. Table (1).

Table 1. Comparison of strategies used to mitigate PCR inhibitors following PEG 6000-mediated virus precipitation

Strategy	Mechanism	Advantages	Limitations	samples	References
DAX-8 resin	Removes humic acids and hydrophobic organic inhibitors	Excellent inhibitor removal; improves RT-qPCR performance	Additional processing; slight viral loss possible	Wastewater, surface water	(13)
Ultrafiltration	removal of low-molecular-weight inhibitors	High viral recovery; suitable for large volumes	Membrane fouling; specialized equipment	Wastewater, environmental water	(18,22)
Magnetic beads	Selective binding of viral nucleic acids; removes contaminants	High nucleic acid purity; automation compatible	Higher cost	Clinical and environmental samples	(8,9)
Sample dilution	Lowers inhibitor concentration of PCR	Simple, rapid and inexpensive	Dilutes nucleic acids, reducing sensitivity	Clinical and wastewater samples	(10,27)
Silica column purification	Adsorption of nucleic acids with sequential washing	Standardized and reproducible	Possible nucleic acid loss; additional cost	Clinical samples, wastewater	(14,16)

7- Limitations and challenges:

Although effective as a low cost, broadly available technique for determining concentration, PEG 6000 precipitation is a variety of limitations, which include co-precipitation of compounds that might interferes with PCR amplification, variable reactivity efficiency depending on viral form and physical properties, an essentially time-consuming with rapid commercially available extraction procedures and the need to carefully optimize PEG concentration, ionic strength, temperatures incubation and centrifugation settings based on the specific characteristic of each samples matrix to ensure reliable and accurate viral detection (27).

Future and prospects:

Standardized and universally recognized PEG 6000 precipitation protocols need to be developed in future research. Furthermore, PEG-based concentration should be combined with advanced purification methods, including ultrafiltration and magnetic bead extraction, to decrease inhibitory substances (28). In the final analysis, these methods should be integrated into automated high-throughput molecular detection systems. Additionally, the role of PEG 6000 in the detection and observation of newly and recurrently identified viral pathogens, including novel respiratory viruses, arboviruses and zoonotic agents, can be further extended for enhancing its utility as a reliable and cost-effective tool for the sensitivity of viral detection in environmental and clinical samples (29,30).

CONCLUSION

Polyethylene glycol 6000 precipitation provides a simple, low-cost and highly efficient method for purifying particles of viruses from clinical and environmental specimens. It greatly enhancing the analytical sensitivity of PCR-based assays by improving the amount of obtainable viral nucleic acid, especially in samples with minimal viral load. This method is widely used for the detection of RNA and DNA viruses and is particularly useful for labs with limited resources. The elements, such as co-precipitation of PCR inhibitors and inter-protocol differences, can impact efficiency, but these limitations may be reduced by optimization techniques and the correct purification procedures. In summary, PEG 6000 still represents a reliable and flexible material for enhancing the accuracy of viral detection and still maintains a key part in the modern era of the molecular diagnostic field and virology studies.

REFERENCES

1-Engstrom-Melnyk J, Rodriguez PL, Peraud O, Hein RC. Clinical Applications of Quantitative Real-Time PCR in Virology. *Methods Microbiol*. 2015;42:161-197. doi:10.1016/bs.mim.2015.04.005.

2- Meng, Z., Guo, S., Zhou, Y. *et al.* Applications of laboratory findings in the prevention, diagnosis, treatment, and monitoring of COVID-19. *Sig Transduct Target Ther* **6**, 316 (2021). https://doi.org/10.1038/s41392-021-00731-z.

3- Christoforou I, Kalatzis A, Siamidi A, Vlachou M, Pispas S, Pippa N. The Ubiquitous Use of Polyethylene Glycol in Pharmaceutical Design and Development: Technological Aspects and Future Perspectives. Nanomaterials (Basel). 2025;15(23):1762. Published 2025 Nov 24. doi:10.3390/nano15231762.

4- Pons Royo, M. del C., & Jungbauer, A. (2025). Polyethylene glycol precipitation: fundamentals and recent advances. Preparative Biochemistry &Biotechnology, 55(8),935–954.https://doi.org/10.1080/10826068.2025.
2470220.

5- Shah N, Hussain M, Rehan T, Khan A, Khan ZU. Overview of polyethylene glycol-based materials with a special focus on core-shell particles for drug delivery application. Current Pharmaceutical Design. 2022 Feb 1;28(5):352-67.

6- Torii S, Oishi W, Zhu Y, et al. Comparison of five polyethylene glycol precipitation procedures for the RT-qPCR based recovery of murine hepatitis virus, bacteriophage phi6, and pepper mild mottle virus as a surrogate for SARS-CoV-2 from wastewater. *Sci Total Environ*. 2022;807(Pt 2):150722. doi:10.1016/j.scitotenv.2021.150722.

7- Bellotti C, Lang K, Kuplennik N, Sosnik A, Steinfeld R. High-grade extracellular vesicles preparation by combined size-exclusion and affinity chromatography. Scientific Reports. 2021 May 18;11(1):10550.

8- Linzner N, Bartel A, Schumacher V, et al. Effective Inhibitor Removal from Wastewater Samples Increases Sensitivity of RT-dPCR and Sequencing Analyses and Enhances the Stability of Wastewater-Based Surveillance. *Microorganisms*. 2024;12(12):2475. Published 2024 Dec 2. doi:10.3390/microorganisms12122475.

9- Yasuura M, Ashiba H, Nomura KI. Evaluation of Viral Collection Efficiency with Antibody-Modified Magnetic Particles by Polymerase Chain Reaction Assay. *Sensors (Basel)*. 2026;26(3):1019. Published 2026 Feb 4. doi:10.3390/s26031019.

10- Smith M. Validating Real-Time Polymerase Chain Reaction (PCR) Assays. *Encyclopedia of Virology*. 2021;35-44. doi:10.1016/B978-0-12-814515-9.00053-9.

11-Pham Le Khanh H, Nemes D, Rusznyák Á, et al. Comparative Investigation of Cellular Effects of Polyethylene Glycol (PEG) Derivatives. *Polymers (Basel)*. 2022;14(2):279. Published 2022 Jan 11. doi:10.3390/polym14020279.

12-Lewis GD, Metcalf TG. Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. Appl Environ Microbiol. 1988 Aug;54(8):1983-8. doi: 10.1128/aem.54.8.1983-1988.1988. PMID: 2845860; PMCID: PMC202790.

13- Hamza, I.A., Leifels, M. Assessment of PCR Inhibitor Removal Methods to Monitor Viruses in Environmental Water Samples: DAX-8 Outperforms Competitors. *Water Air Soil Pollut* **235**, 20 (2024). https://doi.org/10.1007/s11270-023-06821-8.

14- Khehra N, Padda IS, Zubair M. Polymerase Chain Reaction (PCR) [Updated 2025 Jul 7]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK589663.

15- Carroll-Portillo A, Coffman CN, Varga MG, Alcock J, Singh SB, Lin HC. Standard Bacteriophage Purification Procedures Cause Loss in Numbers and Activity. *Viruses*. 2021;13(2):328. Published 2021 Feb 20. doi:10.3390/v13020328.

16-Kleiner M, Hooper LV, Duerkop BA. Evaluation of methods to purify virus-like particles for metagenomic sequencing of intestinal viromes. *BMC Genomics*. 2015;16(1):7. Published 2015 Jan 22. doi:10.1186/s12864-014-1207-4.

17- Pons Royo, M. del C., & Jungbauer, A. (2025). Polyethylene glycol precipitation: fundamentals and recent advances. *Preparative Biochemistry &Biotechnology*, *55*(8),935–954. https://doi.org/10.1080/10826068.2025.2470220.

18-Baral, R.; Nwaubani, D.A.; Solomon, T.; Sherchan, S.P. Assessing Virus Concentration Methods for Norovirus and SARS-CoV-2 Detection in Wastewater. *Environments* **2026**, *13*,86.https://doi.org/10.3390/environments13020086.

19-Fu MX, Larralde O, Mayne R, Kean K, Reid K, Andersson M, Golubchik T, et al. Use of polyethylene glycol precipitation and ultracentrifugation to enhance the sensitivity of hepatitis B virus DNA detection. J Clin Virol. 2025 Jun;178:105802. doi: 10.1016/j.jcv.2025.105802. Epub 2025 May 3. PMID: 40339521.

20- Yu L, Tang Z, Sun Y, et al. A polyethylene glycol enhanced ligation-triggered self-priming isothermal amplification for the detection of SARS-CoV-2 D614G mutation. *Talanta*. 2023;262:124711. doi:10.1016/j.talanta.2023.124711.

21-Sapula SA, Whittall JJ, Pandopulos AJ, Gerber C, Venter H. An optimized and robust PEG precipitation method for detection of SARS-CoV-2 in wastewater. *Sci Total Environ*. 2021;785:147270. doi:10.1016/j.scitotenv.2021.147270.

22-Farkas K, McDonald JE, Malham SK, Jones DL. Two-Step Concentration of Complex Water Samples for the Detection of Viruses. *Methods Protoc*. 2018;1(3):35. Published 2018 Sep 10. doi:10.3390/mps1030035.

23- Pellegrinelli, L.; Castiglioni, S.; Cocuzza, C.E.; Bertasi, B.; Primache, V.; Schiarea, S.;et al. Evaluation of Pre-Analytical and Analytical Methods for Detecting SARS-CoV-2 in Municipal Wastewater Samples in Northern Italy. *Water* **2022**, *14*, 833. https://doi.org/10.3390/w14050833.

24-Cheshomi N, Alum A, Smith MF, Lim ES, Conroy-Ben O, Abbaszadegan M
. 2025. Viral concentration method biases in the detection of viral profiles in wastewater. Appl Environ Microbiol 91:e01339-24.
https://doi.org/10.1128/aem.01339-24.

25-Kalarikkal SP, Prasad D, Kasiappan R, Chaudhari SR, Sundaram GM. A cost-effective polyethylene glycol-based method for the isolation of functional edible nanoparticles from ginger rhizomes. *Sci Rep*. 2020;10(1):4456. Published 2020 Mar 10. doi:10.1038/s41598-020-61358-8.

26- Pawar SD, Keng SS, Tare DS, et al. A virus precipitation method for concentration & detection of avian influenza viruses from environmental water resources & its possible application in outbreak investigations. *Indian J Med Res*. 2019;150(6):612-619. doi:10.4103/ijmr.IJMR_1697_18.

27- Shieh YS, Wait D, Tai L, Sobsey MD. Methods to remove inhibitors in sewage and other fecal wastes for enterovirus detection by the polymerase chain reaction. J Virol Methods. 1995 Jul;54(1):51-66. doi: 10.1016/0166-0934(95)00025-p. PMID: 7559857.

28- Zhang, J.; Zhou, C.; Tan, M.; Cao, Y.; Ren, Y.; Peng, L. Optimization and Characterization of PEG Extraction Process for Tartary Buckwheat-Derived Nanoparticles. *Foods* **2024**, *13*,2624.https://doi.org/10.3390/foods13162624.

29- Mahony JB. Detection of respiratory viruses by molecular methods. *Clin Microbiol Rev*. 2008;21(4):716-747. doi:10.1128/CMR.00037-07.

30-Alexander MR, Rootes CL, van Vuren PJ, Stewart CR. Concentration of infectious SARS-CoV-2 by polyethylene glycol precipitation. *J Virol Methods*. 2020;286:113977. doi:10.1016/j.jviromet.2020.113977.