



Emerging Importance of Viral Transport Media in High-Throughput Sequencing Fidelity for Genomic Analysis

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The impact of Viral Transport Media (VTM) components on the efficacy and accuracy of high-throughput Next-Generation Sequencing (NGS) techniques is a subject of paramount importance in the field of viral genomics and diagnostics. VTMs play a pivotal role in preserving viral samples, and their composition can profoundly influence the quality and reliability of NGS results. This study explores the intricate relationship between VTM components and NGS outcomes, with a focus on optimizing diagnostic precision and the efficiency of NGS in viral genomics. Through comprehensive investigation, this research sheds light on the critical implications of VTM composition for enhancing viral genomic analysis, streamlining diagnostic processes, and bolstering public health responses. The findings have far-reaching consequences, including the potential for personalized medicine, rapid detection of emerging viral threats, and the improvement of public

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health surveillance. Standardized protocols and ongoing technological advancements are essential in harnessing the full potential of NGS for genomics and metagenomics, marking a significant step toward more effective viral disease management and outbreak response.

Keywords: Viral transport media; next-generation sequencing; metagenomics; sequencing accuracy; NGS library preparation.

1. INTRODUCTION

In the global landscape, clinical microbiology, molecular diagnostics, and, more specifically, next-generation sequencing (NGS) have emerged as pivotal areas of focus in the contemporary era. NGS, in particular, holds great promise in the clinical diagnosis of viral and bacterial infections, surpassing conventional diagnostic methods like culture testing and acid-fast bacilli staining (AFS) in terms of sensitivity [1,2]. NGS has demonstrated its effectiveness in detecting various viral pathogens, including emerging strains and co-infections during infections [3]. Additionally, it has proven its utility in identifying non-tuberculous mycobacteria (NTM), fungi, and other prevalent bacterial infections [4]. Furthermore, metagenomic sequencing, a subset of NGS, has exhibited accelerated diagnostic capabilities, outperforming traditional methodologies in promptly identifying infections [5]. Metagenomics also provides a hypothesis-independent approach to profiling microbial communities' taxonomy and function, eliminating the need for cultivating microorganisms in controlled laboratory settings [6]. Clinical metagenomics enables comprehensive pathogen identification and characterization of indigenous microbiota associated with different diseases [7], thus enhancing our understanding of sample microbiomes and their role in disease management. This study delves into how the constituents of basic viral transport media can affect NGS outcomes.

In clinical diagnostics, especially during viral infections, Viral Transport Media (VTM) is commonly used to preserve and transport clinical samples containing viruses such as respiratory viruses, viral conjunctivitis, oral viral infections, and reproductive tract swab samples for viral suspicion. VTMs can significantly impact subsequent processes, including molecular assays and NGS. VTMs may contain additives or components that could interfere with NGS procedures. Contaminants or inhibitors present in Viral Transport Medium (VTM) can affect

enzymatic reactions during library preparation and sequencing, potentially leading to biased or inaccurate sequencing results.

For instance, according to CDC protocols, VTM compositions typically include Gentamicin and Amphotericin B as preservatives [8]. While these preservatives help preserve viral entities in collected samples, they may have adverse effects, especially at higher concentrations, when attempting to detect and characterize other microbial pathogens using NGS. Gentamicin, an aminoglycoside antibiotic, is added to VTM to prevent bacterial contamination in samples that may contain both viral and bacterial agents. Gentamicin is effective against various Gram-negative bacteria but does not work against chlamydia and gonorrhea infections [9]. Amphotericin B, a commonly used antifungal medication, is effective against various fungal species. However, its mode of action involves disrupting fungal cell membranes, potentially interacting with lipids and cell membranes of other constituents in the sample, including viral nucleic acids. Dosage considerations are crucial to inhibit fungal growth while minimizing potential adverse effects on non-fungal components [10].

2. IMPLICATIONS

The implications of the study on the impact of viral transport media (VTM) components on next-generation sequencing (NGS) outcomes are significant and can be summarized as follows:

Enhanced NGS Accuracy: Understanding how VTM components influence NGS results can lead to the development of improved protocols for viral genomic analysis. Researchers can optimize VTM formulations to minimize biases and inaccuracies in sequencing, thus enhancing the accuracy of NGS-based diagnostic tests.

Streamlined Diagnostic Workflows: By identifying VTM formulations that are compatible with NGS, clinical laboratories can streamline their diagnostic workflows. This can lead to faster and more reliable identification of infectious pathogens, which is critical for timely patient care, especially during disease outbreaks.

Improved Surveillance and Research: NGS has revolutionized our ability to monitor and study all type infections as well as differential diagnosis. Knowing how VTMs impact NGS results can help researchers and public health agencies improve surveillance efforts, track the evolution of etiologies, and respond effectively to emerging infectious diseases.

Cost Efficiency: Optimizing VTM formulations for NGS can lead to cost efficiencies in diagnostic testing. By reducing the need for retesting or confirmatory assays due to inaccurate results, healthcare systems can allocate resources more effectively.

Broader Applications: Beyond clinical diagnostics, the findings can have implications for broader applications of NGS, including environmental monitoring, metagenomic studies, and microbial community profiling. Researchers can make informed choices about VTMs in various fields where NGS is employed.

Public Health Impact: Accurate and timely detection of pathogens is crucial for public health interventions and disease control. Improving the reliability of NGS-based diagnostics through VTM optimization can have a direct impact on public health outcomes.

Regulatory Considerations: Regulatory agencies may consider incorporating VTM recommendations into guidelines for diagnostic testing to ensure the accuracy and reliability of NGS-based assays. Standardized transport medium formulations and protocols could become a regulatory requirement.

Education and Training: Healthcare professionals and laboratory personnel may need training and education on the proper selection and use of VTMs in conjunction with NGS. Understanding the implications of VTM choices is essential for ensuring the quality of diagnostic results.

This study's implications extend to improving the accuracy and efficiency of NGS-based genomic analysis, with potential benefits for healthcare, research, and public health efforts. By optimizing VTM components and protocols, we can enhance our ability to diagnose, monitor, and respond to acute and severe infections effectively.

3. VIRAL TRANSPORT MEDIA EFFECTS ON NGS

Understanding the theoretical underpinnings of how VTM components affect NGS outcomes is crucial for optimizing protocols, enhancing diagnostic accuracy, and advancing the field of viral genomics. This knowledge informs the development of standardized practices and technology to improve the efficiency and reliability of NGS-based pathogen identification and characterization [11]. In the context of viral genomics and Next-Generation Sequencing (NGS), the theoretical basis regarding the impact of Viral Transport Media (VTM) components on sequencing efficacy and accuracy is multifaceted:

Viral Sample Preservation: VTMs are crucial for preserving viral samples during collection and transportation. Their constituents, such as buffers, preservatives, and stabilizers, may directly influence the integrity and quality of viral nucleic acids, which are essential for accurate NGS outcomes [12].

Inhibitory Effects: Certain VTM components might contain substances that can inhibit or interfere with enzymatic reactions during the NGS process. These inhibitors can impede the library preparation or sequencing steps, leading to biased or inaccurate results.

Nucleic Acid Integrity: The components of VTMs can impact the stability and integrity of total genomic content present in the sample. Suboptimal VTM compositions might cause degradation or alterations in the viral RNA/DNA, affecting the reliability of sequencing data [13].

Optimization for Sequencing Efficiency: Understanding the influence of VTM components on NGS outcomes allows for the optimization of VTM formulations. Identifying or modifying VTM constituents can enhance sequencing efficiency, ensuring high-quality data for better diagnostic accuracy.

Standardization and Protocol Development: Research into VTM impact on NGS provides a basis for standardizing VTM compositions and protocols. Standardized VTMs can lead to consistent and reproducible results across different laboratories and settings.

Diagnostic and Clinical Relevance: Exploring VTM's influence on NGS is critical for improving

diagnostic precision and clinical relevance. Accurate NGS results from properly preserved samples aid in identifying viral and / or microbial pathogens, understanding their genomic variations, and guiding appropriate therapeutic interventions. The analysis of concerned genome/genome in question /genome of interest is very crucial.

Technological Advancements: Research in this area paves the way for technological improvements in VTM formulations, NGS library preparation, and sequencing protocols. This enables the development of more robust and reliable methods for genomic analysis.

4. FUTURE PROSPECTS

The future prospects in the field of viral genomics, particularly in the context of Viral Transport Media (VTM) and next-generation sequencing (NGS), are promising and multifaceted. Improved NGS techniques with optimized VTMs can pave the way for personalized treatments tailored to individual patients, increasing treatment efficacy and minimizing side effects. The ability to quickly detect and characterize novel viruses using NGS and VTMs will be crucial in responding to emerging infectious diseases and preventing global outbreaks. Advanced NGS capabilities, coupled with standardized VTMs, will bolster public health surveillance systems, allowing for real-time monitoring of viral strains and other potentially pathogenic(infectious) and non-pathogenic(non-infectious) strains of different microbial origin thus facilitating early intervention. NGS can expedite the discovery of potential drug targets in viral genomes, expediting the development of antiviral drugs and vaccines. Metagenomics, supported by optimized VTMs, will provide a deeper understanding of complex microbial ecosystems, including the human microbiome. Rapid identification and characterization of viral agents through NGS and VTMs will be vital for preventing bioterrorism and ensuring biosecurity. International cooperation and data sharing will continue to be critical in viral genomics research, with standardized VTM protocols promoting data comparability. The integration of machine learning and artificial intelligence into NGS data analysis, backed by high-quality VTMs, will enable more efficient interpretation of sequencing data. NGS and optimized VTMs will play a role in monitoring viruses and other microbes in environmental reservoirs, contributing to disease prevention

efforts. Ongoing education and training programs will be essential to ensure proficiency in NGS techniques and VTM selection among healthcare professionals and researchers. Regulatory bodies are likely to establish and refine guidelines for VTM use in NGS-based diagnostics and research, promoting standardized practices and data quality. The future prospects in viral genomics, particularly concerning VTMs and NGS, encompass personalized medicine, rapid response to emerging threats, improved public health surveillance, therapeutic development, deeper microbial insights, biosecurity, global collaboration, AI integration, environmental monitoring, education, and regulatory advancements. These prospects hold the potential to reshape healthcare, research, and public health strategies in the years ahead.

5. CONCLUSION

This study underscores the significant influence of Viral Transport Media (VTM) components on the accuracy and reliability of next-generation sequencing (NGS) outcomes in the microbiome and genomic analysis. Understanding the implications of VTM choices is crucial for various domains, including clinical diagnostics, research, and public health. By optimizing VTM formulations and protocols, we can enhance the precision of NGS-based pathogen detection, streamline diagnostic workflows, improve public health preparedness, advance research efforts, and potentially influence regulatory guidelines. Moreover, education and training in VTM selection and usage are essential for ensuring the quality of NGS results. This study contributes valuable insights into the critical interplay between VTMs and NGS, ultimately promoting more effective genomic analysis with broader implications for healthcare and scientific advancements.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Laura M, Filkins Robert, Schlaberg. Metagenomic Applications for Infectious Disease Testing in Clinical Laboratories; 2021. DOI: 10.1007/978-3-030-62155-1_7

2. Qingqing, Wang, Qing, Miao, Jue, Pan., Wenting, Jin, Yuyan, Ma., Yao, Zhang., Yumeng, Yao., Yi, Su., Yingnan, Huang., Bing, Li., Mengran, Wang., Na, Li., Sishi, Cai., Yu, Luo., Chunmei, Zhou., Honglong, Wu., Bijie, Hu. The clinical value of metagenomic next-generation sequencing in the microbiological diagnosis of skin and soft tissue infections.. International Journal of Infectious Diseases; 2020. DOI: 10.1016/J.IJID.2020.09.007
3. Chendi Jing, Hongbin Chen, Liang Yong, Ying Zhong, Qi Wang, Li Lifeng, Shijun Sun, Yifan Guo, Ruobing Wang, Jiang Zhi, Hui Wang. Clinical Evaluation of an Improved Metagenomic Next-Generation Sequencing Test for the Diagnosis of Bloodstream Infections. Clinical Chemistry; 2021. DOI: 10.1093/CLINCHEM/HVAB061
4. Hanna Jerome, Callum Taylor, Vattipally B, Sreenu, Tetyana Klymenko, Ana da Silva, Filipe, Celia, Jackson, Chris, Davis, Shirin Ashraf, Eleri Wilson-Davies, Natasha Jesudason, Karen Devine, Lisbeth, Harder, Celia, Aitken, Rory, Gunson E, Thomson. Metagenomic next-generation sequencing aids the diagnosis of viral infections in febrile returning travellers. Journal of Infection; 2019. DOI: 10.1016/J.JINF.2019.08.003
5. Cui-Lin Shi, Peng Han, Peijun Tang, Meng-Meng Chen, Zhi-Jian Ye, Mei-Ying Wu, Jie Shen, Hai-Yan Wu, Zhu-Qing, Tan, Xin Yu, Guan-Hua Rao, Jian-Ping Zhang. Clinical metagenomic sequencing for diagnosis of pulmonary tuberculosis. Journal of Infection; 2020.3 DOI: 10.1016/J.JINF.2020.08.004
6. Heleen Delbeke, Saif Younas, Ingele Casteels, Marie Joossens. Current knowledge on the human eye microbiome: a systematic review of available amplicon and metagenomic sequencing data. Acta Ophthalmologica; 2021. DOI: 10.1111/AOS.14508
7. Schlager Robert, Schlager Robert, Laura M, Filkins. Clinical metagenomics for infection diagnosis; 2019. DOI:10.1016/B978-0-12-801496-7.00004-6
8. Kirkland PD, Frost MJ. The impact of viral transport media on PCR assay results for the detection of nucleic acid from SARS-CoV-2. Pathology, 2020;52(7):811-814.
9. Petersen J, Dalal S, Jhala D. Criticality of in-house preparation of viral transport medium in times of shortage during COVID-19 pandemic. Laboratory Medicine. 2021;52(2):e39-e45.
10. Holohan C, Hanrahan S, Feely N, Li P, O'Connell J, Moss C, Lee GU. Influence of viral transport media and freeze-thaw cycling on the sensitivity of qRT-PCR detection of SARS-CoV-2 nucleic acids. Nanoscale. 2021;13(37):15659-15667.
11. Fitzpatrick AH, Rupnik A, O'Shea H, Crispie F, Keaveney S, Cotter P. High throughput sequencing for the detection and characterization of RNA viruses. Frontiers in microbiology. 2021;12: 621719.
12. Nakamura S, Yang CS, Sakon N, Ueda M, Tougan T, Yamashita A, Nakaya T. Direct metagenomic detection of viral pathogens in nasal and fecal specimens using an unbiased high-throughput sequencing approach. PloS one. 2009;4(1):e4219.
13. Bull RA, Adikari TN, Ferguson JM, Hammond JM, Stevanovski I, Beukers A. G, Deveson IW. Analytical validity of nanopore sequencing for rapid SARS-CoV-2 genome analysis. Nature communications. 2020;11(1):6272.

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