Optimized Viral Stock Purification Enhances Applications in NGS Performance Testing

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INTRODUCTION

High-sensitivity molecular assays for replacing in vivo testing can detect a broad range of viruses, but often also capture residual host genomic material. To address this, we explore the role of purified viral stocks in adventitious agent testing (AAT) performance qualification and highlight the importance of rigorous stock characterization to support sensitive detection methods.

This study evaluates how viral stock purification enhances the utility of reference materials for AAT developers. We optimized gradient ultracentrifugation and enzymatic digestion to produce high-titer, purified viral stocks with reduced host genomic content, supporting more accurate assay performance testing (Figure 1).

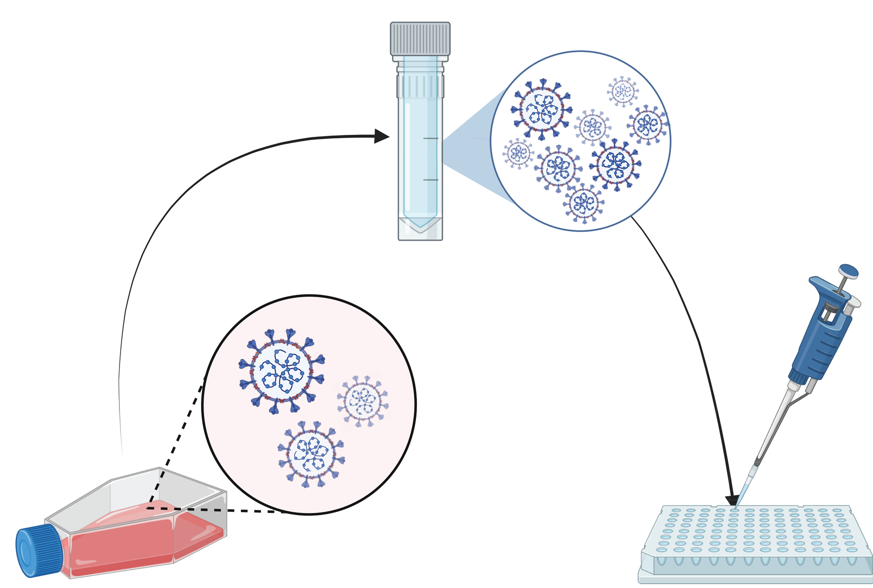

FIGURE 1: Viral cultures (left) are processed to prepare viral stocks (top). The viral stocks are then measured for quality and analyzed for use in diverse testing applications (right) such as adventitious agent testing.

METHODS

High-titer viral stocks of Influenza B virus (IBV), Minute Virus of Mice (MVM), and Herpes simplex virus type 2 (HSV-2) were produced. Purification via gradient ultracentrifugation and enzymatic digestion enriched viral particles and minimized host cell carryover.

Crude and purified viral stocks underwent infectivity assays, virus/host quantification via ddPCR, and NGS validation (Figure 2) to assess purity and suitability for AAT applications.

DNA and RNA were extracted, converted to double-stranded cDNA, and prepared for Illumina MiSeq short-read NGS. Taxonomic classification via k-mer analysis assessed the impact of purification on sample composition and utility for NGS-based AAT.

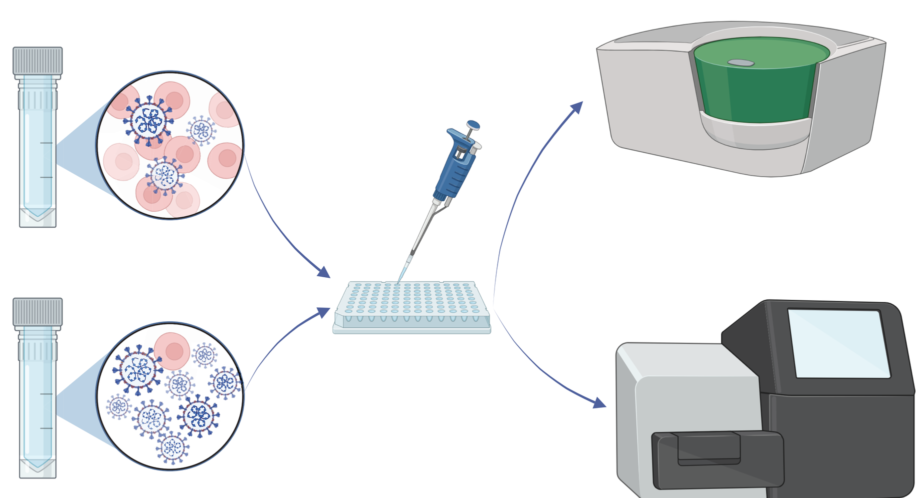

FIGURE 2: Viral stocks produced from both crude lysate (top) and purification procedures are processed for analysis on both droplet digital PCR (ddPCR) and next-generation sequencing (NGS).

RESULTS

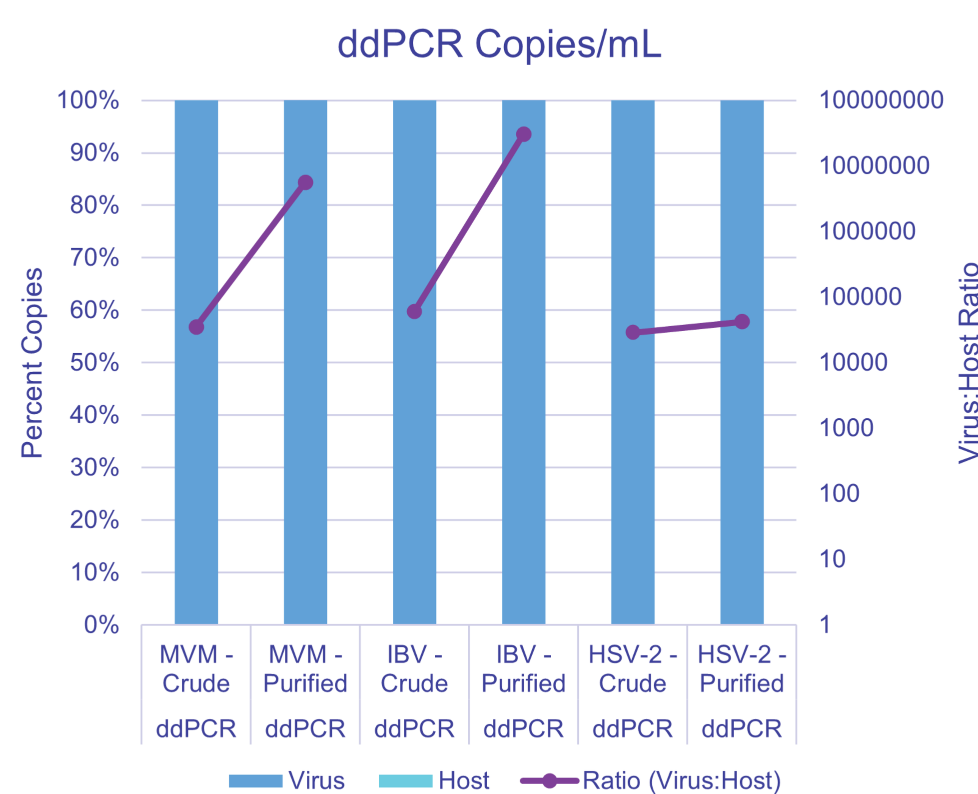

FIGURE 3: Measured copy numbers of host and virus via ddPCR. The stacked bar chart represents the percentage of virus and host copies per mL while the overlaid line chart reveals the ratio of virus to host copies.

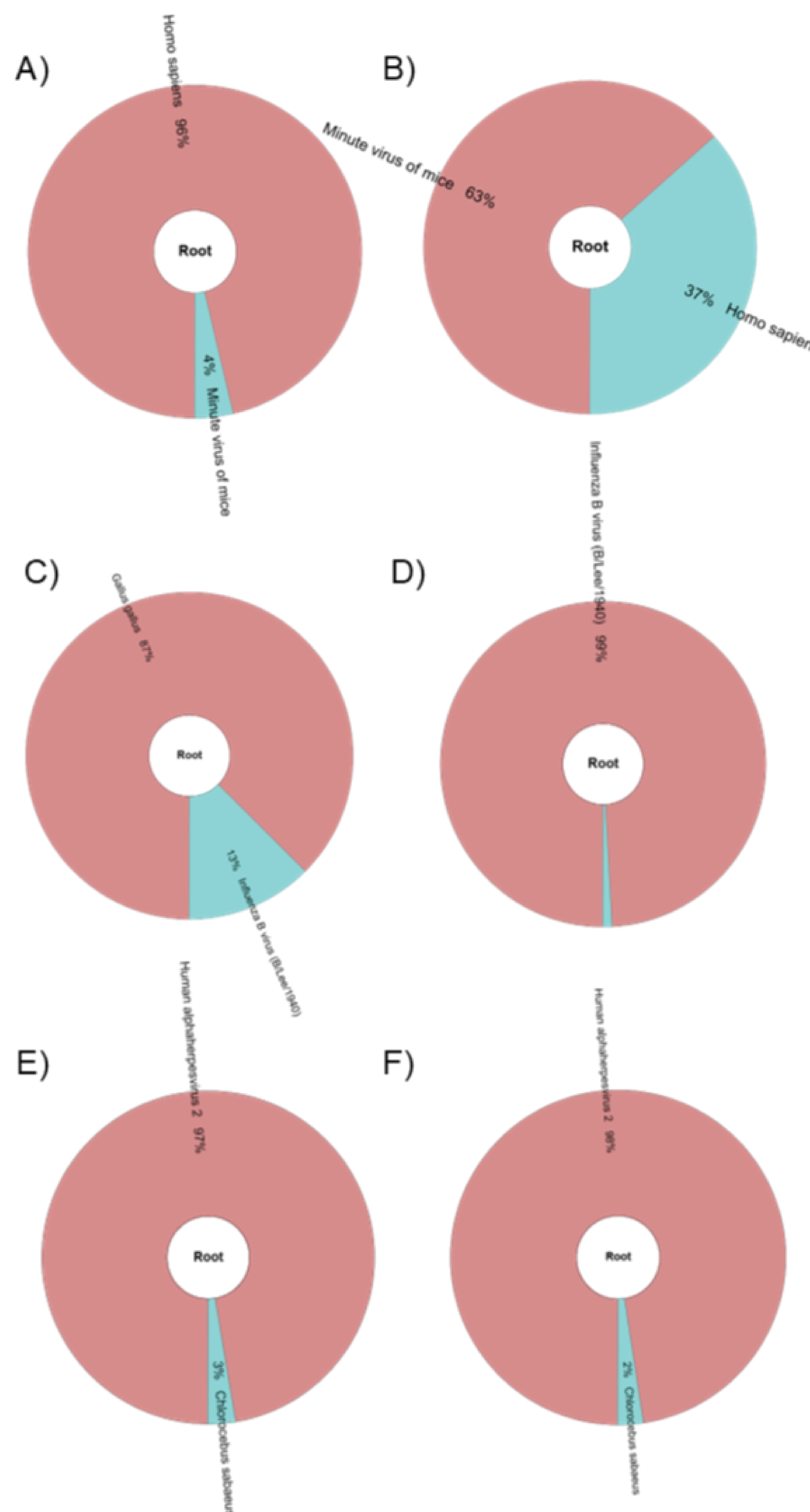

FIGURE 4: Krona plots of total nucleic acid NGS reads that passed quality filtering/read trimming and classified with KRAKEN2 K-mer classification for MVM (A) crude and (B) purified viral stock, IBV (C) crude and (D) purified viral stock, as well as HSV-2 (E) crude and (F) purified viral stock.

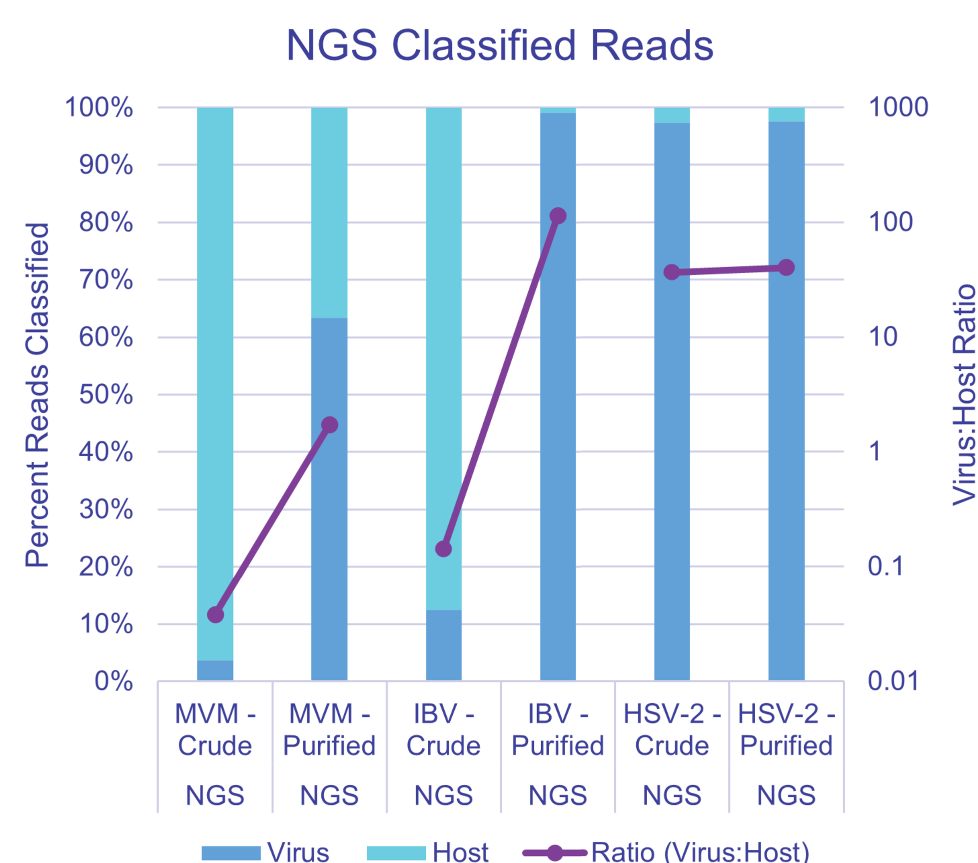

FIGURE 5: Measured classified reads of host and virus via NGS metagenomics analysis. The stacked bar chart represents the percentage of virus and host classified reads while the overlaid line chart reveals the ratio of virus to host reads.

RESULTS

Quantification by ddPCR confirmed a significant increase in the proportion of viral to host genomic copies following purification for IBV, MVM, and HSV-2 stocks (Figure 3).

Metagenomic NGS analysis showed stock purification improved virus-to-host read ratios, though the degree of improvement varied between IBV, MVM, and HSV-2 despite comparable titers (Figures 4 & 5). While ddPCR and NGS results correlated, the magnitude of virus-to-host ratio improvement differed by test method, highlighting technology-specific sensitivity to purification effects (Figure 6).

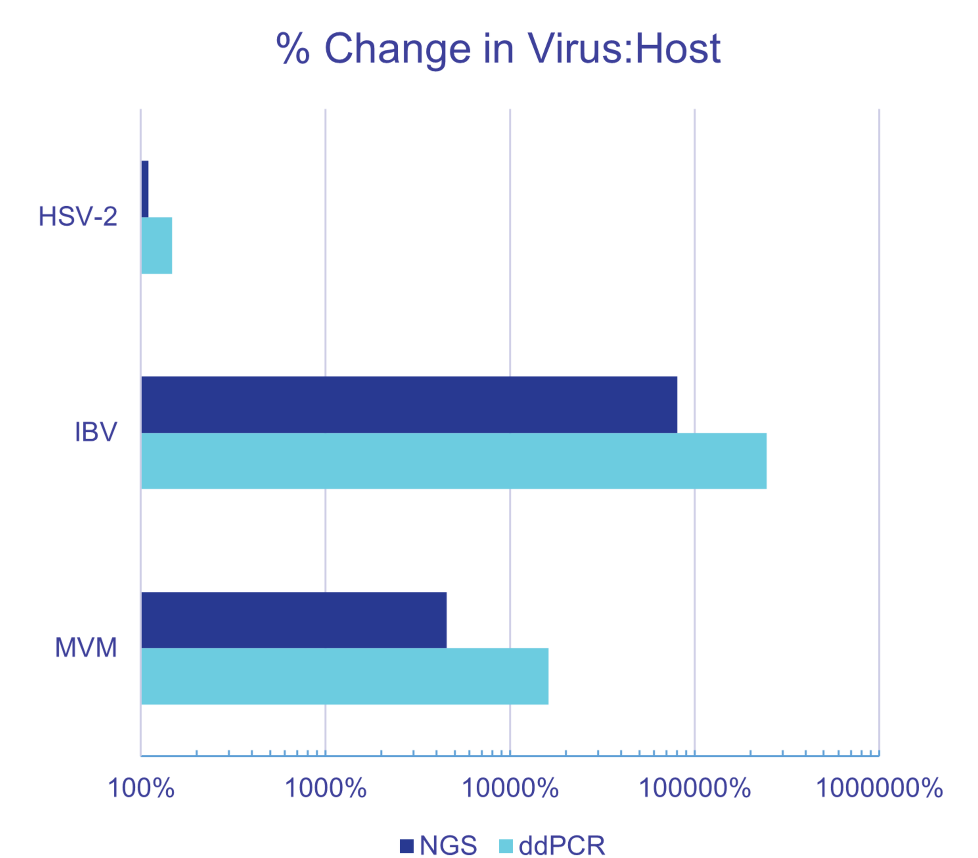

FIGURE 6: Measure of the increase in the ratio of virus to host abundance between crude lysate and purified stocks for both ddPCR and NGS test methods. Results are grouped by virus type: HSV-2, IBV, and MVM.

CONCLUSION

NGS-based adventitious agent testing is challenged by residual host genomic material in viral stocks. Optimized purification reduces host background while maintaining high viral titers, simplifying bioinformatics analysis and improving assay reliability. Spike-in studies remain essential to validate assay performance in complex matrices.

Minimizing residual spike-in material enhances consistency across testing modalities and simplifies bioinformatics workflows (Figure 7). While improvements may vary by virus type, the significant benefit observed with MVM and IBV supports purification as a recommended practice for reference stock preparation.

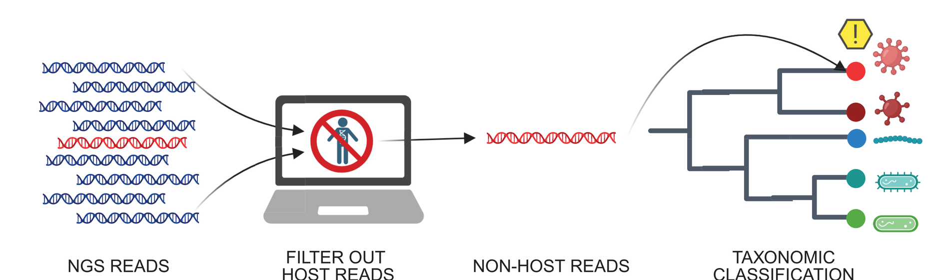

FIGURE 7: A high-level schematic of next-generation sequencing (NGS) bioinformatics for adventitious agent testing (AAT). Raw NGS data is processed for classification, often including a dehosting step to filter expected host reads from the sample matrix.

ACKNOWLEDGEMENTS

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