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A rapid assay to conduct in-vitro polyomavirus DNA replication

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Traditionally, the HIRT extraction method using phenol and chloroform, is employed to selectively extract low-molecular weight DNA to conduct polyomavirus DNA replication assays. Moreover, DNA replication results obtained with this approach are typically inconsistent between triplicate specimens. To hasten the procedure of polyomavirus DNA replication assay and to enhance reproducibility, we compared the DNA quality and yield using Qiagen Spin Column technology and the HIRT extraction method. CV-1 cells were transfected with SV40 DNA, cells were harvested at days 2, 4 and 6, and DNA was extracted using the Qiagen Spin Column and the HIRT extraction methods. Southern hybridization was performed using a 32P labeled linear full-length SV40 DNA. Viral DNA replication was quantitated using the BioRad phosphorimager and results obtained with the two procedures were compared. Additionally, B-cells and Rat2 cells were spiked with 500 ng of JC polyomavirus linear DNA and processed as described above, except 32P labeled full-length linear JCV DNA was employed in the Southern hybridization step. Southern blot analysis revealed consistent SV40 and JCV DNA recovery using the Qiagen Spin Column technology, and SV40 DNA replication was consistent between triplicate specimens at all time points. In addition, SV40 DNA recovery was greater using the Qiagen Spin Column technology as judged by Southern blot analysis. The traditional HIRT extraction method employed to conduct polyomavirus DNA replication assays takes approximately 24 hr, whereas the Qiagen Spin Column technology reduces the time required to obtain good quality DNA to less than one hr. These modifications to the assay will improve the determination of polyomavirus DNA replication activity, while reducing exposure of the investigator to toxic organic compounds.