Performance Validation of BK Virus Nanogen v.2.0 Assay: Method Comparison to Nanogen v.1.0
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Introduction: Reactivation of BK polyomavirus (BKV) in renal transplant patients has a strong association with the development of allograft nephropathy in 1-5% of patients, with subsequent graft failure in approximately 50%. Quantitative molecular detection of BK viremia has been established as an effective screening and monitoring tool in renal transplant recipients. As there is no FDA cleared assay or international standard for BKV, several analyte specific reagents (ASRs) are available for clinical use. To this end, we compared the performance characteristics of two currently available ASRs. Materials and Methods: DNA from EDTA plasma samples was extracted using the MagnaPure Total Nucleic Acid kit. Standards and samples were run using Nanogen v.2.0 and v.1.0 ASRs with probes and primers targeting BKV. Real-time PCR analysis was performed on the ABI 7500 Sequence Detection System. Linearity was assessed by serial dilutions of Nanogen BKV standard. The limit of detection was determined by probit analysis of 12 replicates each of 8 serial dilutions. Within-run precision (%CV) was determined with 12 sample replicates. Sixty-five samples within the dynamic range of both assays were compared. Evaluation of bias included linear regression analysis and determination of the mean difference and standard deviation of assay results. Results: The comparison of the results between assay systems showed 94% qualitative agreement (61/65). Three samples that were undetectable by v.1.0 had detectable viral DNA in v.2.0; one sample detected by v.1.0 was undetectable by v.2.0. All four values were below or near the limit of quantification. The assay was determined to be linear over a range of 862 to 6.5 x 10^7 copies/ml. Terminal dilution studies demonstrated 95% detection at 862 copies/ml, as determined by probit analysis. The mean difference between assay results was -0.138 log10 copies/ml (SD+/-0.292). The CV near the limit of detection was 3% at approximately 3.8 log10 copies/ml and 15% at 3.0 log10 copies/ml. Conclusions: The Nanogen v.2.0 assay demonstrates comparable laboratory performance to the v.1.0 assay. Correlation between assays shows a clinically insignificant slight negative bias for v.2.0 with a lower limit of detection. Precision at values near the limit of detection is within acceptable limits. v.2.0 of BKV ASR has the advantages of less background drift and inclusion of an internal control to detect extraction failures or inhibition.