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Evaluation of a BK virus viral load assay using the QIAGEN Artus BK Virus RG PCR test

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ABSTRACT

Background: Viral load testing for BK Virus (BKV) has become the standard of care for the diagnosis of infection and monitoring of therapy of kidney transplant patients infected with BKV. However, there are currently no FDA-approved BKV quantification assays and no standardization among available tests.
Objective and study design: This study evaluated the performance of the Artus BK Virus RG PCR (RUO) assay (QIAGEN) for accuracy, linearity, precision, analytical sensitivity, specificity, and correlation with a referral laboratory test in patient samples.
Results: Linear regression analysis of the quantitative results demonstrated a linear range of quantification from 192 to 194 million (2.28 to 8.29 $\log_{10}$) DNA copies/mL and a coefficient of determination (R^2) of 0.994. A dilution series demonstrated a limit of detection and a limit of quantification of 2.00 $\log_{10}$, and 2.30 $\log_{10}$ copies/mL (>95% positivity rate), respectively. The precision of the assay was highly reproducible among runs with coefficients of variance (CV) ranging from 0.2% to 7.0%. A comparison of 34 matched samples showed a good agreement (R^2 = 0.983) between the Artus BK test and the referral laboratory results, with an average positive bias (0.39 $\log_{10}$ copies/mL). Genotyping analysis using large-T antigen sequences demonstrated that 90% of the positive samples were BKV type I, and that there was no significant difference in quantification between the referral laboratory and Artus BK Virus tests.
Conclusions: The Artus BK Virus RG PCR test is a reliable and sensitive assay for BKV DNA quantification as compared to the referral laboratory test.

1. Introduction

BK virus (BKV) is a common double-stranded DNA virus in the *Polyomaviridae* family that latently infects up to 90% of adults.[1] Renal transplant recipients are at risk of developing organ dysfunction, including tubulointerstitial lesions known as polyomavirus-associated nephropathy (PVAN), frequently leading to graft failure in 15–80% of patients.[2,3] BKV reactivation in bone marrow of transplanted patients may result in hemorrhagic cystitis. Treatment usually involves a reduction or change in immunosuppressive therapy.

The clinical utility of BKV viral load testing in predicting BKV-associated nephropathy (BKVAN) has greatly improved patient management.[4,5] Although the viral load cutoff associated with nephropathy is not well established, a small number of studies[6] have shown that plasma BKV loads >10^4 copies/mL have a predictive value greater than 80%, while the absence of viremia and/or viruria rules out the diagnosis of BKVAN.[7] Quantitative measurements of BKV DNA have also shown that decreasing virus levels over time correlate with response to treatment and improved outcome. Moreover, screening guidelines currently recommend that renal-transplant patients are screened every 3 months up to 2 years post-transplant or when allograft dysfunction occurs, or when allograft biopsy is performed.[6]

Virus levels are commonly quantified by direct measurement of BKV DNA in plasma using real-time PCR amplification technologies. However, providing clinicians with accurate results has been challenging because most methods are laboratory-developed, and there are no international standards to compare among the different tests. In this study we evaluated the performance of the Artus BK Virus RG PCR assay, coupled with automated extraction and PCR set-up. We show that this assay is comparable to the referral laboratory BKV test, providing additional testing options for clinical laboratories.

2. Objectives

This study evaluated the accuracy, linearity, precision and specificity as well as the ability to detect and quantify the BK viral loads

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by the Artus BK Virus RG PCR assay and the Rotor-Gene Q system, using blinded plasma specimens tested by a referral laboratory.

3. Study design

3.1. Samples and performance evaluation panels

A total of 59 consecutive plasma samples submitted to our clinical laboratory for BKV testing were prospectively collected during 2009. Samples were de-identified and stored frozen at −70 °C, until further analysis. No clinical information was available on these patients. All samples had undergone BKV viral load testing by Viracor-IBT referral laboratory (Lee's Summit, MO) prior to validation. The study was approved by the IRB Committee at Weill Cornell Medical College.

The NATtrol BK Linearity Panel (ZeptoMetrix, Buffalo, NY) was used to evaluate accuracy, reproducibility, linearity and sensitivity. The six-member panel, formulated with intact BKV (subtype Ia) particles, was prepared in a serum protein matrix that mimics the composition of true clinical samples, spanning the clinically significant range (1×10^4 to 1×10^8 BKV copies/mL) for BK infected individuals. A 5×10^2 copies/mL Acrometrix BK panel member (Benicia, CA) was additionally utilized for the limit of quantification (LOQ). For determining the limit of detection (LOD), the 1×10^4 copies/mL Zeptometrix samples were further diluted in BKV-negative plasma and analyzed in three to six replicates. Panel members were quantified by the manufacturer's assay, targeting the BKV large T-antigen (LTA) gene and calibrated against a commercial purified BKV DNA. The within-run and between-run precision was determined, using three replicates of high- and low-viral load commercial and patient samples analyzed on three different days. Cross-reactivity (specificity) was determined using high-viral load samples tittered in-house (CMV, EBV, HIV, HCV and HBV) or by Viracor (HHV-7). HSV-1, HSV-2, HHV-6, HHV-8 and JC viruses were obtained from Advanced Biotechnologies (Columbia, MD) and VZV from the Centers for Disease Control [CDC] (Atlanta, GA).

3.2. Artus BK Virus RG PCR assay

BKV DNA was extracted from 200 μL of plasma using the QIAcube/QIAamp DNA Extraction reagents. The DNA was eluted into a final volume of 50 μL, of which 15 μL were added to the master mix containing the primers and probes, *Taq* polymerase, magnesium chloride, and buffers in a total volume of 25 μL. The BKV viral load was then measured by a quantitative TaqMan real-time PCR assay, using the Artus BK Virus RG PCR kit and the Rotor-Gene Q thermal cycler (QIAGEN, Germantown, MD). The kit contains primers and probes specifically amplifying a 274-bp region of the BKV VP2 and VP3 genes, covering all available BKV sequences, including the rare strains of types II, III, and IV, and an artificial 280-bp internal control sequence that is co-amplified with the BKV DNA (QIAGEN, unpublished communications). The quantification standards, QS1–QS4 (10 to 10,000 copies/mL), used for the BKV quantification are also included with the kit.

3.3. Viracor-IBT referral laboratory assay

The BK viral load test by Viracor-IBT is a laboratory-developed quantitative real-time PCR (QPCR) assay. The assay amplifies multiple BK viral targets and an internal inhibition control. The assay dynamic range used for this validation was reported to be between 500 copies/mL and 10^{10} copies/mL. The assay was designed to detect all strains of BKV, using primers and probes specific for all known BKV strains based on similarity search algorithms. Additionally, no cross reactivity was detected when tested against adenoviruses, and other viruses (http://www.viracor.com/).

Table 1
Limit of detection of the Artus BK Virus RG PCR test using plasma samples.

Log_{10} BKV copies/mL	No. of positive samples/no. tested	%Positive
2.30	20/20	100.0
2.00	19/20	95.0
1.70	8/10	80.0
1.40	3/10	30.0

The lowest titer Zeptometrix linearity panel member (1×10^4 copies/mL) was serially diluted in BKV-negative plasma and analyzed in 10–20 replicates and the percentage of positive results determined.

3.4. BKV sequence analysis for genotyping

PCR amplification of a 732-bp region of the BKV LTA gene was performed using the Dunlop reference strain as described by Hoffman et al.[8] DNA sequencing was performed, using the Big Dye Terminator Chemistry on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Genotype assignments (I through IV) were performed by comparison to the Dunlop strain (V01108) as described by Luo et al.[9] Genotype assignments of I and IV versus II or III, were initially made by the SNP at position 4339, and further identification of a genotype II versus III was accomplished with an additional SNP at position 4270.

3.5. Statistical methods

The correlation of log_{10} BKV loads in plasma and Zeptometrix validation specimens was determined by the least-square regression model using Microsoft Excel 2003 (Redmond, WA). A log10 difference of 1.0 was used as the cutoff for the determination of concordance between the two assays as previously described.[1,6,8] The within-run and between-run precision of the QPCR method for quantifying BKV was expressed by the coefficient of variation (CV). Agreement between the Artus BK Virus RG-PCR and the Viracor-IBT test was determined using a Bland–Altman plot of all samples (34 samples). A positivity rate of 95% for all dilution replicates was used for determining the LOD. The LOQ was defined as the lowest dilution with an SD within 0.15 log_{10}.[10]

4. Results

4.1. Test performance characteristics

Testing of a commercial linearity panel across the range of detection from 192 to 194 million (2.28 to 8.29 log_{10}) DNA copies/mL demonstrated an excellent agreement between the expected and observed viral loads with a coefficient of determination (R^2) of 0.994 (Fig. 1). The assay was linear with a slope approximating one (1.1) across six logs of detection. We did not have a high-titer specimen to measure the linear range beyond 8.00 log_{10}.

When a larger number of inter-assay dilutions were tested to assess the limit of detection, the concentration at which BKV DNA was detected in at least 95% of the replicates was 2.00 log_{10} copies/mL (100 copies/mL) (Table 1). The LOQ was 2.30 log_{10} copies/mL (200 copies/mL) based on an SD less than or equal to 0.15 log_{10} copies/mL (Table 2).

Reproducibility analysis using low and high patients samples in six replicates had a mean viral load of 2.05 log_{10} copies/mL (SD = 0.15) and 5.96 log_{10} copies/mL (SD = 0.06), respectively. The commercial validation specimens yielded BKV viral load means of 2.28 log_{10} copies/mL (SD = 0.03) and 8.29 log_{10} copies/mL (SD = 0.09) for the low and high controls, respectively.