Supplemental Material

Nucleic Acid Extraction

Nucleic acid extraction was performed using the EZ1 Virus Mini Kit, v2.0 on the EZ1 Advanced XL instrument (both from Qiagen, Valencia, CA). All extractions were carried out according to the manufacturer's recommendations. Extractions were performed using 200µl of patient plasma or urine and were eluted into 60µl of elution buffer. A plasmid containing sequence from the green fluorescent protein gene from *A. victoria* flanked by BK V3a Forward and V3a Reverse primer sequences (Supplementary Table 1) was added to each extraction to ensure adequate nucleic acid recovery and the absence of PCR inhibitors.

Quantitative, Real-Time PCR

BKV DNA quantitation was performed using the LightCycler Taqman Master mix on the LightCycler 2.0 instrument (both from Roche Applied Science, Indianapolis, IN). Reactions were performed at a total volume of 20µl, and 10µl of purified nucleic acids were added to each reaction. The primer and probe concentrations in the final reaction mixture are listed in Supplemental Table 1. Cycling conditions were the following: 95°C for 10 min; 45 cycles of 95°C for 15 sec, 60°C for 30 sec, and 72°C for 40 sec. The ramp rate was set at 20°C/sec for each temperature change. Detection was performed in the green (530nM) and yellow (560nM) channels at 72°C. Negative and no template controls, as well as high positive and low positive BKV controls (Zeptometrix, Buffalo, NY) were tested on each run. In addition, each run contained a five point standard curve

using pBKV34-2 plasmid (ATCC, Manassas, VA) containing the whole genome of the Dunlop reference. This assay has a linear range of 1,000 – 60,000,000 copies/ml plasma or urine.

Long-Range PCR and Sequencing

BKV whole genomes were amplified by long-range PCR with four primer sets (Supplemental Table 2) using the SequalPrep Long PCR Kit (Invitrogen, Grand Island, NY). The 20 µl reactions were comprised of 2 µl 10x Reaction Buffer, 0.4 µl DMSO, 1 µl 10x Enhancer A, 1.8 U enzyme, and 250nM of each primer. Cycling conditions were: 94°C for 2 min; 5 cycles of 94°C for 10 sec, 60°C for 30 sec, and 68°C for 5 min; 35 cycles of 94°C for 10 sec, 60°C for 30 sec, and 68°C for 5 min/+20 sec/cycle; followed by a final extension at 68°C for 10 min. The complete BKV genomes were acquired by primer walking and were deposited in GenBank (KF055891-KF055893).

Supplemental Table 1. BKV real-time PCR primers and probes.

Name	Sequence (5`-3`)	Concentration	Position[a]	Gene
V3a Forward	CCTTACCCAATTTCCTTTTTGCT	1000nM	2458-2480	VP1
V3a Reverse	ATACATAGGCTGCCCATCCAC	1000nM	2512-2532	VP1
V3a Probe	FAM-TGGGTTCTCCTGTTTATA-BHQ+	500nM	2490-2507	VP1
GFPCal560 Probe	CalFluor560-TACCTGAGCACCCAGTCCGCCCT-BHQ-1	250nM	NA	NA

[a]Relative to the Dunlop reference sequence (GenBank accession no. V01108)
FAM: Fluorescein amidite
Primers were obtained from Eurofins MWG Operon (Huntsville, AL)
Probes were obtained from Biosearch Technologies, Inc. (Novato, CA)

Supplemental Table 2. BKV long-range PCR primers.

Primer name	Primer Set	Sequence (5`-3`)	Position[a]	Gene
BK_HR	1	TCTGGGTTTAGGAAGCATTCTACCTCT	1700-1727	VP1
BK_JF	1	AATTTAAATGAGGACCTAACCTGTGGAAATCT	1855-1886	VP1
BK_KR	2	AGGCCTCTCCACTGTTGTGTTCCAG	2387-2411	VP1
BK_OF	2	CTGCTTTTGTATAAGCCACTTTTAAGCTTGTGT	2690-2722	Large T antigen
BK_OR	3	TGAATGGAAGGAAAGGCTGGATTCTGA	2987-3013	Large T antigen
BK_QF	3	ATTAGCAGTAGCAACAAGGTCATTCCACTTTGTA	3065-3098	Large T antigen
BK_WR	4	CTTGCCTGCTTTGCTGTGTATAC	4316-4338	Large T antigen
BK_ZF	4	GCTTTAGATCTCTGAAGGGAGTTTCTCCAATT	4640-4671	Small t antigen

[a]Relative to the Dunlop reference sequence (GenBank accession no. V01108)
Primers were obtained from Integrated DNA Technologies (Coralville, IA)