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Comparative evaluation of laboratory developed real-time PCR assays and RealStar® BKV PCR Kit for quantitative detection of BK polyomavirus

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A B S T R A C T

Background: Quantitative, viral load monitoring for BK virus (BKV) by real-time PCR is an important tool in the management of polyomavirus associated nephropathy in renal transplant patients. However, variability in PCR results has been reported because of polymorphisms in viral genes among different subtypes of BKV, and lack of standardization of the PCR assays among different laboratories. In this study we have compared the performance of several laboratory developed PCR assays that target highly conserved regions of BKV genome with a commercially available, RealStar® BKV PCR Kit.
Method: Three real-time PCR assays (i) VP1 assay: selected from the literature that targets the major capsid protein (VP1) gene (ii) VP1MOD assay: VP1 assay with a modified probe, and (iii) BKLTA assay: newly designed assay that targets the large T antigen gene were assessed in parallel, using controls and clinical specimens that were previously tested using RealStar® BKV PCR Kit (Altona Diagnostics GmbH, Hamburg, Germany). Nucleic acid from all samples were extracted using the QIA symphony virus/bacteria kit on an automated DNA extraction platform QIA symphony SP (Qiagen). Primer and probe concentration, and reaction conditions for laboratory developed assays were optimized and the limit of detection of different assays was determined. Positive control for laboratory developed BK assays was prepared through construction of a plasmid carrying respective amplicon sequences.
Results: The 95% detection limit of VP1, VP1MOD and BKLTA assays were 1.8×10^2, 3×10^3 and 3.5×10^2 genomic copies/ml, respectively, as determined by Probit regression analysis of data obtained by testing a dilution series of a titered patient specimen, using RealStar® BKV PCR Kit. The inter-assay and intra-assay, coefficient of variations of these assays using calibrated, plasmid standards were <1%. All assays, including the RealStar® BKV PCR assay, were highly specific when tested against a panel of external proficiency specimens containing both BK and JC viruses. All assays, except the VP1MOD assay determined BK viral load in proficiency specimens within the same log values. With reference to results obtained by RealStar® BKV PCR assay, the sensitivity and specificity of different assays tested in 116 serum specimens submitted for BK viral load assay were 91% and 97% for VP1 assay, 88% and 97% for VP1MOD assay, and 97% and 98% for BKLTA assay, respectively. BK Viral load in positive specimens determined by various assays was highly correlated ($R^2 > 0.97$), based on linear regression analysis.
Conclusions: The performance characteristics of the newly designed, BKLTA assay were highly comparable to RealStar® BKV PCR assay, and can be used for routine detection and viral load monitoring of BKV in a cost-effective manner.

1. Introduction

The BK polyomavirus, also called BK virus (BKV) is ubiquitous and highly prevalent in humans but is thought to cause disease mainly in immunocompromised individuals (Nickeleit and Singh, 2015). BKV is a non-enveloped, double-stranded DNA virus of the

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polyomaviridae family, which sub-clinically infects 82%–90% of the general population worldwide (Mandell et al., 2010; Pinto and Dobson, 2014). The primary infection is usually asymptomatic and occurs in early childhood followed by latent infection in the kidneys as well as other anatomical sites. Reactivation of BKV and subsequent viral shedding in urine occurs in 0–20% of asymptomatic immunocompetent individuals and in 20–60% of immunocompromised patients (Dalianis and Hirsch, 2013). BKV reactivation is associated with significant morbidity in immunocompromised patients such as polyomavirus nephropathy (PVN) in renal allograft recipients and early and late onset of hemorrhagic cystitis (HC) in BMT recipients (Han et al., 2014; Nickeleit and Singh, 2015). The risk of developing these conditions has increased as a consequence of the introduction of new potent immunosuppressants such as tacrolimus and mycophenolate in kidney transplantation and new myeloablative conditioning regimens for patients undergoing hematopoietic stem cell transplantation (HSCT) (Borni-Duval et al., 2013; Dalianis and Ljungman, 2011).

The BKV predilection for the genitourinary tract is of particular concern in renal transplant patients where virus reactivation is associated with nephropathy that occurs in up to 10% of kidney allograph recipients (Hirsch et al., 2005; Nickeleit and Singh, 2015). Hemorrhagic cystitis occurs in 9–31% of all stem cell transplantation (SCT) recipients and is associated with urinary tract obstruction, renal dysfunction and renal failure, and longer hospital stays (Han et al., 2014).

The diagnostic challenges in predicting the risk of the development of PVN and HC are significant (Nickeleit and Singh, 2015). BKV can be detected in urine by polymerase chain reaction (PCR), or by urine cytology to observe the presence of decoy cells, which is believed to be indicative of BK virus infection. BKV PCR on both urine and plasma has been proven superior to cytology for screening BK virus associated nephropathy with much higher sensitivity, specificity, and positive and negative predictive values (Bechert et al., 2010). Renal biopsy to observe viral replication in the tubular epithelial cells with large intranuclear inclusions is also used in the diagnosis of nephropathy, but a false-negative result in up to 30% can be observed (Mandell et al., 2010; Pinto and Dobson, 2014). Current clinical screening recommendations include PCR based BKV screening in urine and plasma as it is known that most cases of clinically significant PVN are preceded by a period of up to months of asymptomatic viruria followed by viremia (Nickeleit and Singh, 2015; Randhawa and Brennan, 2006). This approach is useful to rule out BKV induced nephropathy as the negative predictive value of BKV PCR in urine and blood approaches 90%. In contrast, the positive predictive value only ranges between 25 and 75%. To improve the positive predictive value of PCR based BKV assays, threshold levels of 10^4 copies/ml for BKV viremia have been advocated (Drachenberg et al., 2004; Nickeleit and Singh, 2015). It may pose a problem in that not all BKV PCR assays are equally sensitive and specific (Nickeleit and Singh, 2015).

In fact, variability in viral load PCR results from different laboratories has been reported which may be partly due to polymorphisms within viral genes of different subtypes of BKV but also due to the of lack of standardization of BKV PCR assays (Buller, 2010; Hoffman et al., 2008). Nevertheless, regular monitoring of BK viral load in blood and urine in transplant patients is recommended for the early detection of BKV replication for pre-emptive therapy and to monitor the course of BKV infection (Nickeleit and Singh, 2015; Randhawa and Brennan, 2006). In this study, we have compared the performance of three laboratory-developed PCR assays that target the highly conserved regions of the BKV genome, based on most recent sequence information, with a commercially available, RealStar® BKV PCR Kit.

2. Materials and methods

2.1. Specimens

One hundred and sixteen serum specimens submitted to the Microbiology and Virology laboratory of BC Children's Hospital between February 2014 to October 2014 were maintained at −80 °C following initial testing for BK virus. To maintain patient anonymity, each sample was coded and all patient identifiers were removed to ensure that personnel involved in this study were unaware of any patient information. Ethics approval was not considered necessary, because studies that involve the retrospective use of anonymous human biological materials are exempted from review by the local, Research Ethics Board of University of British Columbia. DNA from 0.2 ml of serum specimens was extracted using the QIAsymphony virus/bacteria kit in an automated DNA extraction platform QIAsymphony SP (Qiagen).

2.2. Controls and standards

A serum specimen, positive for BK virus with a viral titre 7.95×10^8 copies/ml was diluted in TE8 buffer (10 mM Tris; 1 mM EDTA; pH 8.0), and used to determine the optimum primer and probe concentration and reaction conditions for laboratory developed tests (LDTs). The same sample was also used to determine the limit of detection of different PCR assays. A BK virus minigene, consisting of amplicon sequences for all in-house PCR assays used in this study was custom made and cloned into the pIDTsmart vector (Integrated DNA Technologies). The plasmid stock (38 ng/μl or 1.55×10^{10} copies/μl) was diluted in TE8 buffer to prepare working stocks, which were aliquoted and maintained at −80 °C. The working stock was further diluted in neonatal calf serum (NCS) to final concentrations of 5×10^5, 5×10^4, 5×10^3, and 5×10^2 copies/ml and subjected to DNA extraction for use as quantification standards for various assays. For clinical validation, samples from external quality assessment programmes (Quality Control for Molecular Diagnostics, QCMD) were used along with clinical specimens. For use as an internal control, an unrelated plasmid (pUC19) was spiked to each specimen prior to extraction to a final concentration of 1.6×10^5 copies/ml.

2.3. Quantitative PCR

Primers and probes (Table 1) for various laboratory-developed tests were designed by using the Primer Express software v3.0.1 (Life Technologies). Initial testing of controls and clinical specimens were performed by RealStar® BKV PCR Kit (Altona Diagnostics GmbH, Hamburg, Germany) according to manufacturer's instructions. For laboratory developed, Taqman real-time PCR assays, 5 μl of sample extract was mixed with 20 μl of a master mix containing 12.5 μl of Taqman Universal PCR Master Mix (Applied Biosystems) and primers and probes to final concentrations shown in Table 1. Thermal cycling was performed in a ABI7500 Fast instrument (Applied Biosystems) with 1 cycle of 95 °C–10 min, followed by 45 cycles consisting of 95 °C–15 s and 60 °C–60 s. BKV quantification standards and a negative control (NCS) were analysed by PCR in parallel with clinical specimens. DNA extraction and PCR inhibition was monitored by an in-house, internal control PCR assay, using the primers and probes shown in Table 1.

2.4. Analysis of performance characteristics of laboratory developed tests

For Probit regression analysis, a BK virus positive patient specimen, titered with the RealStar® BKV PCR Kit was serially diluted to 10, 50, 100, 200, 500 and 1000 genomic copies/ml, and 8 repli-

Table 1
Description of primers and probes used in this study.

Assay	Target gene	Genome position*	Primer/probe	Sequence (5′-3′)	Working concentration	Reference
VP1	VP1	1564–1582	Forward	ATGGCCCCAACMAAAAGAA	1 μM	(Mitui et al., 2013)
		1625–1644	Reverse	TAGTTTTGGCACTTGCACGG	1 μM	
		1592–1616	Probe	FAM-TCCAGGGGCAGCTCCCAAAAAGCC-BHQ1	0.2 μM	
VP1MOD	VP1	1564–1582	Forward	ATGGCCCCAACMAAAAGAA	1 μM	(Mitui et al., 2013)
		1625–1644	Reverse	TAGTTTTGGCACTTGCACGG	1 μM	
		1587–1610	Probe	FAM-AGAGTGTCCAGGGGCAGCTCCCAA-BHQ1	0.2 μM	
BKLTA	Large T antigen	4336–4358	Forward	CAGGCRAGDGTTCTATTACTAAATACAG	1 μM	This study
		4393–4421	Reverse	AAAARAGAAAGGTAGAAGACCCTAAAGAC	1 μM	
		4364–4384	Probe	FAM-CTAAGAAACTGGTGTAGATCA-MGB	0.2 μM	
Internal Control	pUC19 plasmid		Forward	TCCCGGCATCCGCTTA	0.3 μM	This
			Reverse	GTGATGACGGTGAAAACCTCTGA	0.3 μM	study
			Probe	FAM-ACAAGCTGTGACCGTCTCCGGGAG-3IABkFQ	0.1 μM	

* With respect to accession number NC_001538.

Table 2
Performance characteristics of different BKV PCR assays.

PCR assay	95% LOD (copy/μl)	Linearity over 10^0–10^9 copies/ml (R squared value)	Intra-assay variation (coefficient of variation%)	Inter-assay variation (coefficient of variation%)
RealStar® BKV	0.71	0.99	3.73	3.94
VP1	0.18	0.98	0.17	0.60
VP1mod	3	0.98	0.44	0.72
BKlta	0.35	0.99	0.26	0.51

cates of each dilution were tested by real-time PCR. The reportable ranges of PCR assays were verified by a linearity study using a titered patient specimen (1.44×10^{11} copy/ml; quantitated using the RealStar BKV PCR Kit), which was serially diluted (10^{11}–10^0 copies/ml) logarithmically. PCR assays were performed in triplicate along with quantification standards. Viral loads, determined by LDTs, were plotted against assigned values in a scatter plot and the R-squared values were calculated by linear regression analysis. For intra-assay variation, six replicates of each of plasmid standards (5×10^5, 5×10^4, 5×10^3, and 5×10^2 copies/ml) were analysed simultaneously. Coefficient of variation (SD/Mean × 100) of C_T values were calculated for each standard and averaged. For inter assay variation, plasmid standards (5×10^5, 5×10^4, 5×10^3, and 5×10^2 copies/ml) were analysed in duplicate, in three independent experiments, performed in different days. Coefficient of variation of C_T values were calculated for each standard and averaged.

2.5. Statistical analysis

Probit regression analysis to determine the 95% detection limit of various BKV PCR assays was performed as described previously (Burd, 2010). Correlation between the results of different assays was determined by Cohen's kappa test. Correlation of viral loads by different methods was performed by linear regression analysis.

3. Results

In this study, serum specimens submitted for quantitative detection of BK virus were first analysed by RealStar® BKV PCR assay, followed by laboratory developed, Taqman assays using three sets of primers and probes (Table 1): (i) VP1 assay, based on a study described by Mitui et al. (Mitui et al., 2013) designed to target the VP1 gene (ii) VP1MOD assay, based on a modification of assay described by Mitui et al. (Mitui et al., 2013) and (iii) BKLTA assay, a newly designed, minor groove binding (MGB) probe-based assay, designed to target the large T antigen gene. VP1 and VP1MOD assays differ by their probe sequences only. The purpose of this modification was to improve PCR efficiency, by using a probe sequence that is closer to the forward primer by distance, without affecting the in-silico specificity of the probe to detect BKV. The in-silico specificity of the probe was ascertained by an alignment (NCBI blast) of 377 BKV nucleotide sequences against, highly related, 807 nucleotide sequences from the genomes of JC polyoma virus and Simian virus 40. The BKLTA amplicon sequence is highly conserved among 342 BKV sequences available in the nucleotide database, while distinct enough from closely related JC polyoma viruses. Representative sequences are shown in Fig. 1. Primers contained mixed bases at various positions because of nucleotide variation in corresponding genomic sequences of different BKV types and subtypes.

Performance characteristics of the laboratory developed, BKV PCR assays were determined with reference to the RealStar® BKV assay. As determined by Probit regression analysis, the lowest concentration (with 95% probability) of viral target detected by VP1 and BKLTA assays were similar (< 500 genomic copies/ml), while the VP1MOD assay had an LOD one log higher than that of other LDTs (Table 2). As described in the Materials and Methods section, a custom made plasmid, containing amplicon sequences for all LDTs was used in various assays as quantification standards. Standard curves prepared for various assays had minimal variation (Fig. 2). Viral loads, determined by all assays were fairly linear, from 10^0 to 10^9 copies/ml, with R-squared values ≥0.98 (Table 2). The intra-assay and interassay variation of C_T values determined by these PCR assays were <0.5% and <0.75%, respectively (Table 2).

For clinical validation, 116 serum specimens were tested for quantitative BKV detection and the results were compared with their previously known results determined by RealStar® BKV PCR assay. PCR inhibition was not noted for any of the specimens irrespective of test methods (data not shown). Based on Cohen's kappa test, the strength of agreement of all LDTs with RealStar® BKV PCR assay was good (kappa value >0.8). However, the BKLTA assay demonstrated highest correlation with a kappa value of 0.935 (95% CI 0.86–1.0). The sensitivity and specificity of different assays were 90% and 96% for VP1 assay, 87% and 96% for VP1MOD assay, and 97% and 98% for BKLTA assay, respectively (Table 3). By linear regression analysis, viral loads determined by BKLTA assay was highly correlated with RealStar® BKV PCR assay ($R^2 = 0.99$) and VP1 assay was highly correlated with VP1MOD assay ($R^2 = 0.99$) (Fig. 3). When tested against an external quality assessment (EQA) panel for BK and JC viruses, all assays, including the RealStar® BKV PCR assay, were highly specific for the detection of BK virus. However, viral

A)

BK Genotype	Accession No.	Sequence	Base position
Target		ATGGCCCCAAC*M*AAAAGAAAAGGAGAGTGTCCAGGGGCAGCTCCCAAAAAGCCAAAGGAACCCGTGCAAGTGCCAAAACTA	1-81
BKV-Ia	JF894228	..........C.....	1583-1663
BKV-Ib1	AB211374	..........C.....	1444-1524
BKV-Ib2	AB301092	..........A.....G	1444-1523
BKV-Ic	AB211377	..........C.....	1436-1516
BKV-II	AB263920	..........C.....	1434-1514
BKV-III	AB211386	..........C.....	1443-1523
BKV-IVb1	AB211390	..........C.....	1354-1434
BKV-IVc2	AB369093	..........C.....	1445-1525
BKV-V	AB211370	..........A.....	1405-1485
BKV-VI	AB211369	..........C.....	1444-1524
JC virus	NC_001699	..........A..... .....C..........T.....	146915-24

B)

BK Genotype	Accession No.	Sequence	Base position
Target		CAGGC*R*AG*D*GTTCTATTACTAAATACAGCTTGACTAAGAAACTGGTGTAGATCAGAGGGAAAGTCTTTAGGGTCTTCTACCTTTCT*Y*TTTT	1-91
BKV-Ia	JF894228	.....A..G.....T....	4350-4440
BKV-Ib1	AB211374	.....A..G.....A.....C....	4211-4301
BKV-Ib2	AB301092	.....G..G.....A.....T....	4211-4301
BKV-Ic	AB211377	.....A..G.....A.....T....	4203-4293
BKV-II	AB263920	.....A..T.....T....	4195-4285
BKV-III	AB211386	.....A..T.....T....	4198-4288
BKV-IVb1	AB211390	.....A..A.....A.....T....	4123-4213
BKV-IVc2	AB369093	.....A..A.....A.....T....	4214-4304
BKV-V	AB211370	.....A..G.....A.....T....	4172-4262
BKV-VI	AB211369	.....A..G.....A.....C....	4211-4301
JC virus	NC_001699	..A.CA.....C.....G..G..TGCA..C.....TACA.....T.T..	4197-4282

Fig. 1. Alignment of BKV PCR amplicon sequences with various types and subtypes of BKV genome. (A) Alignment of VP1 and VP1MOD PCR amplicon sequence (B) Alignment of BKLTA PCR amplicon sequence. Underlined sequences indicate the forward primer, probe and reverse primer, respectively (from left). Shaded sequence in (A) shows the probe sequence for VP1MOD PCR assay. Dots indicate homology with the sequence in the top rows. Mixed bases in the primers sequences are shown in bold and italicized font.

Table 3
Clinical accuracy of laboratory developed assays compared to RealStar BKV PCR assay.

	VP1	VP1MOD	BKLTA
Total samples	116	116	116
Total positive	31	30	32
Total negative	85	86	84
Observed agreement (kappa; 95% CI)	94.8% (0.87; 0.77–0.97)	94.8% (0.87; 0.77–0.97)	97.4% (0.935; 0.86–1.0)
Sensitivity	90	87	97
Specificity	96	96	98
Positive predictive value	90	90	94
Negative predictive value	96	95	99

Table 4
Analysis of a QCMD proficiency panel for BKV and JCV by RealStar BKV and Laboratory developed PCR assays.

Samples	Virus	QCMD (copy/ml)	RealStar BKV (copy/ml)	VP1 (copy/ml)	VP1MOD (copy/ml)	BKlta (copy/ml)
BK12-01	BKV	508	312	70	84	201
BK12-02	BKV	5260	2501	2743	726	3645
BK12-03	BKV	21,086	24,877	22087	6412	21384
BK12-04	JCV	0	0	0	0	0
BK12-05	JCV	0	0	0	0	0
BK12-06	BKV	79	17	0	41	96
BK12-07	Neg	0	0	0	0	0
BK12-08	BKV	54,325	40,060	36,527	29,789	47,840
BK12-09	JCV	0	0	0	0	0
BK12-10	JCV	0	0	0	0	0
BK12-11	JCV	0	0	0	0	0
BK12-12	JCV	0	0	0	0	0

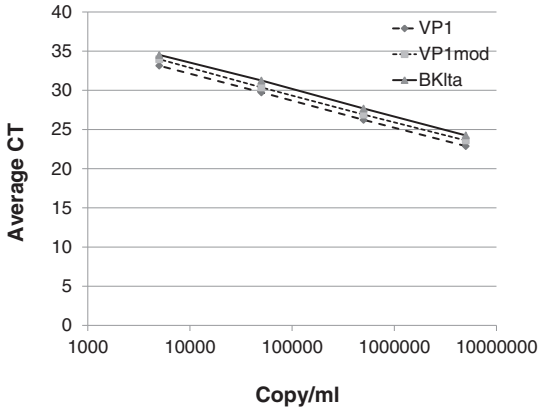

Fig. 2. Standard curves used to determine BK viral loads by various laboratory developed PCR assays. A custom made, pIDTsmart plasmid harbouring amplicon sequences of all laboratory developed PCR assays used in this study was diluted to final concentrations of 5×10^5, 5×10^4, 5×10^3, and 5×10^2 copies/ml, subjected to DNA extraction (to mimic patient specimens) and analysed by various PCR assays. C_T values were plotted against the number of plasmid copies/ml.

loads determined by RealStar® BKV PCR assay and BKLTA assay provided the closest values to EQA expected results (Table 4).

4. Discussion

Molecular diagnostic assays for infectious diseases, in particular real-time PCR, have become an indispensable part of clinical microbiology laboratories. Because of their higher sensitivity and faster turn-around time, PCR assays for many infectious pathogens are now considered as the gold standard, replacing traditional culture methods (Nolte and Caliendo, 2010). Moreover, PCR assays have enabled laboratories to perform testing for an infectious agent that is either difficult to culture or require a lengthy incubation period to grow in the laboratory. BK polyomavirus is one such pathogen, for which traditional methods such as culture, antigen detection and serology has little or no role in routine diagnosis. PCR is the method of choice for detection and quantification of BKV in most diagnostic microbiology laboratories. At present, there is no FDA approved nucleic acid amplification test available for the detection and quantification of polyomaviruses (Buller, 2010). However, several commercial tests are available including the RealStar® BKV PCR Kit that was used in this study, which is a CE-IVD marked *in-vitro* diagnostic test for detection and quantification of BKV specific DNA. Apart from commercial assays, a large number of LDTs with varying chemistries and platforms have been described in the literature (Bista et al., 2007; Dumonceaux et al., 2008; Funahashi et al., 2010; Iwaki et al., 2010; McNees et al., 2005; Mitui et al., 2013; Moret et al., 2006). Designing PCR assays for BKV is particularly challenging because of the diversity of nucleotide sequences among different types and subtypes of BKV (Buller, 2010; Hoffman et al., 2008). Several previous studies reported inter-assay variability on BK viral loads, discrepant PCR results, and false negative PCR results arising from nucleotide mismatch between BKV types and primers and probes used in PCR assays (Buller, 2010; Hoffman et al., 2008; Luo et al., 2008). Again, non-satisfactory results with BKV positive specimens were seen in a survey conducted by the College of American Pathologists (CAP) that included data from 48 laboratories that used both laboratory-developed tests and commercial tests (Buller, 2010). It is likely that commercial tests, for which primer and probe sequences remain unknown to the user, could also be affected by the same problem. The clinical implication of poor efficiency of PCR because of nucleotide mismatches between oligonucleotides and their targets is significant. Inaccurate viral load determined above or below the cutoff values (according to current guidelines) for viruria (10^7 copies/ml) or viremia (10^4 copies/ml) may affect the decision for a renal biopsy for renal transplant recipients (Buller, 2010). Careful selection of amplification target is therefore highly important for uniform, quantitative detection of various types and subtypes of BKV. With rapid advancement in DNA sequencing technology, and accumulation of sequence data from newer variants of BKV, it is also important to update PCR assays in the light of latest sequence information in the databases.

In this study, we performed in-silico analyses on several, previously published amplicon sequences for BKV PCR (Funahashi et al., 2010; Hoffman et al., 2008; Iwaki et al., 2010), and selected an assay (VP1 assay) described by Mitui et al. (Mitui et al., 2013). Our choice

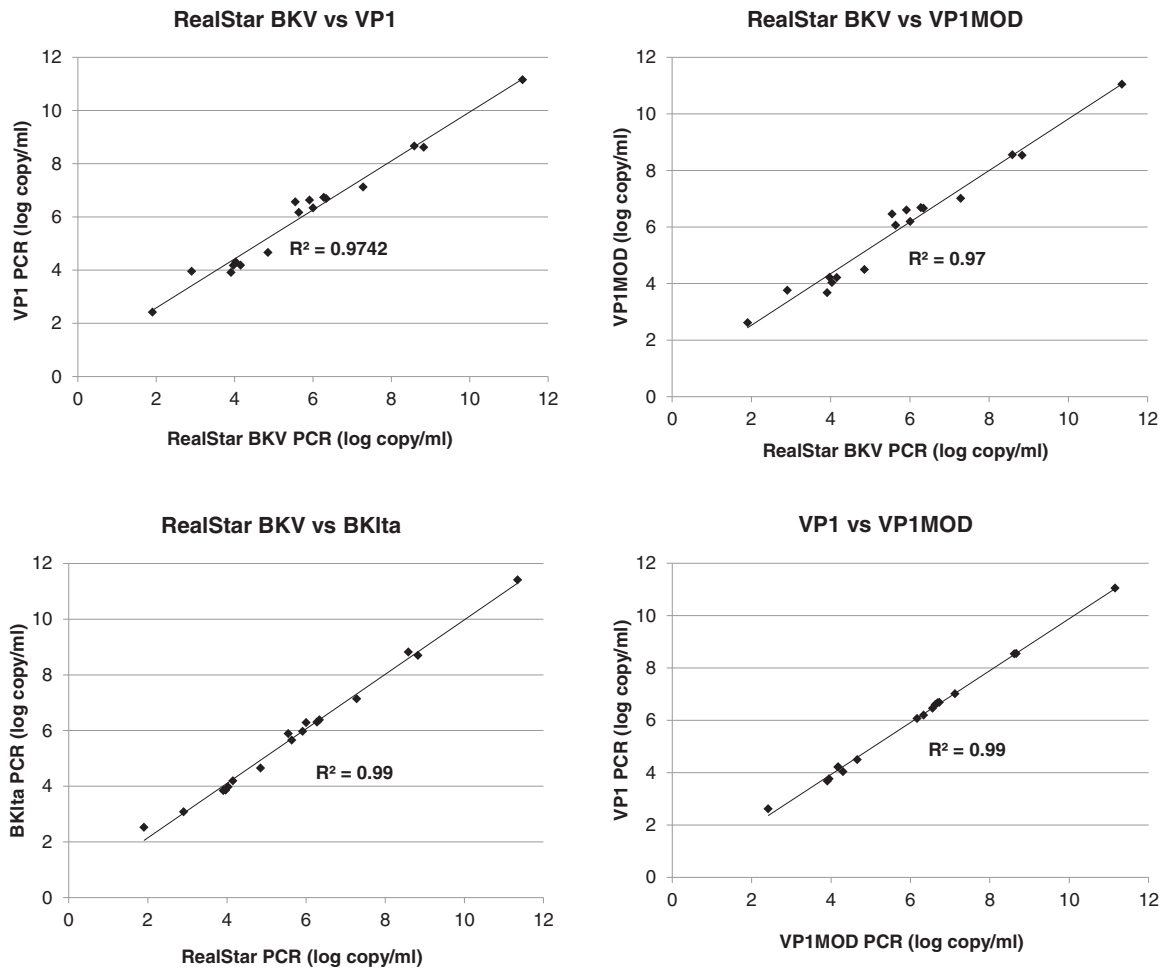

Fig. 3. Correlation of viral loads determined by various PCR assays. Log values of viral loads determined by various PCR assays in serum specimens positive for BKV were plotted against each other in a scatter plot and the coefficient of determination (R^2) was determined by linear regression analysis.

was based on the high sequence conservation of its target sequence amongst different BKV types and subtypes, as well as other factors that affect PCR efficiency such as amplicon length, annealing temperature, and compatibility with Taqman real-time PCR chemistry. In order to achieve better PCR efficiency, we developed a modified assay (VP1MOD) through a minor modification to the probe sequence only. The modified probe is closer to the forward primer, and thus has a lower penalty score compared to the unmodified assay according to the primer design software, Primer Express software v3.0.1. In addition to these assays, we also designed a new, MGB-probe based assay targeting the large T antigen gene. The performance characteristics of all, laboratory-developed assays were compared to that of RealStar® BKV PCR assay, which was considered the gold standard.

We noted that the modification of probe sequence did not change the clinical accuracy of VP1 assay, while the analytical sensitivity (95% LOD) of the modified assay was poor compared to other assays (Tables 2 and 3). The analytical sensitivity, linearity and precision (inter-assay and intra-assay variability) of the VP1 assay and BKLTA assay were comparable to each other. Compared to RealStar® BKV PCR assay, both VP1 and BKLTA assays showed better analytical sensitivity and precision. On the other hand, the clinical accuracy (sensitivity and specificity) of BKLTA assay was superior to those of the VP1 and VP1MOD assays in a comparison-of-methods study with RealStar® BKV PCR assay as the gold standard. Again, the BKLTA assay agreed more with the RealStar® BKV PCR assay both in terms of BKV detection and viral loads (Table 3 and Fig. 3). The BKLTA assay and the RealStar® BKV PCR assay were also more accurate in predicting viral loads, than the other two assays in an EQA panel for BK and JC viruses, although the specificities of all assays were not different.

A limitation of this study is that it was performed at a single site, and may not represent the full range of BKV viral sequences circulating elsewhere. Laboratories considering in-house methods should confirm the ability of the assay to provide quantitative results with their local strains. Another limitation is that urine samples were not extensively assessed in this study although it seems unlikely that PCR results would vary much by specimen type because PCR was performed on extracted specimens.

Overall, our results show that both the RealStar® BKV PCR assay and the BKLTA assay performed similarly in clinical validation. However, the analytical performance of the BKLTA assay was slightly better than RealStar® BKV PCR assay. Commercial assays are far more expensive in terms of costs associated with reagents and consumables. For clinical laboratories with the capacity to perform in-house methods, the new BKLTA assay described in this study may serve as a valuable new tool to provide accurate quantitative BKV detection.

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