Evaluation of the *Sentosa SA* BKV quantitative PCR test in urine and plasma samples

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Abstract

Introduction: Ninety percent of the adult population is seropositive for BK virus. Primary infections are generally asymptomatic. Viral reactivation is a major concern for immunocompromised patients, especially the renal transplant population where persistent infection leads to nephropathy in 1-10% of cases increasing the risk of kidney allograft dysfunction or loss. Viral assays are usually done by laboratory developed tests, resulting in extensive inter-laboratory variability. This presents a challenge given reliance on precise viral titers for clinical decisions. For example, 10^4 copies/mL is widely used as the established viremia cut off for risk of nephrotoxicity. We evaluated the performance of the Vela Dx system, automating nucleic acid extraction and setup for quantitative assays on the RotoGene QMDX. The test targets the VP2/VP3 region of the BK viral genome and covers all isolates registered in GenBank. Linearized plasmids are used for calibration standards and positive controls, while the tobacco mosaic virus is used as internal control. The linear range of the assay is 500 to 10^8 copies/mL with a lower limit of detection of 187 copies/mL. **Materials and Methods**: The AcroMetrix BK virus panel was used for linearity determinations in plasma and urine. Accuracy was assessed from results of CAP proficiency samples. Precision was determined by testing replicates of AcroMetrix low and high positive controls. Analytical sensitivity was determined from replicate dilutions of a high positive control. **Results** Analysis of the 5 concentrations across the reportable range in plasma showed linear quantitative results (R^2=0.9875) that remained linear (R^2=0.9987) after spiking into normal urine. Copies/mL in plasma were 0.32 $\log_{10}$ higher than expected panel values while those in urine were 0.22 $\log_{10}$ higher. Results of the last 8 positive CAP proficiency tests varied between 2.4 to 4.18 $\log_{10}$. The Sentosa results were on average to 0.70 $\log_{10}$ higher than those of the CAP overall median results. Low and high control aliquots ran daily over 11 days showed excellent repeatability, with coefficient variances below 3.38% and 1.00% respectively, while CV values for reproducibility in urine were 10.66% and 6.84%. Nine of 10 (90%) aliquots with low titers at a calculated concentration of 200 copies/mL ran over 5 days tested positive. **Conclusion:** The results validate the reportable range, accuracy, precision and sensitivity of the Sentosa BKV quantitative assay for BK virus. Its wider adoption might alleviate problems with inter-laboratory variability associated with the multitude of laboratory developed tests that are currently in use.

Methods

Calibration: Quantitation standards (1x10^1 1x$10^2$1x$10^3$1x10^4 copies/mL) were purchased from Vela Dx. A standard curve was generated by running the 4 quantitation standards for each new lot of BKV PCR kit reagents received.

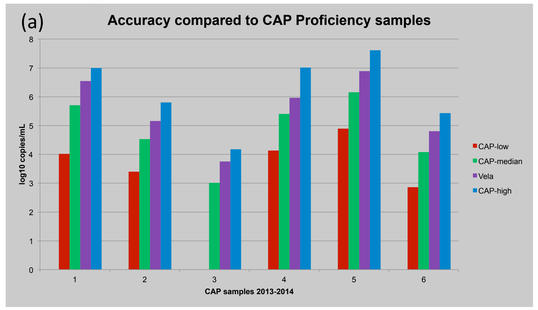
Linearity: BKV panels purchased from AcroMetrix (Life Technologies) where used to determine linearity for both plasma and urine matrices. The panel consists of BKV dilutions 5E2 to 5E6 copies/mL made in normal human EDTA plasma. The panel was used unmodified for linearity in plasma. For linearity in urine, the panel was admixed 1:1 in BKV negative urine to generate dilutions of 2.5E2 to 2.5E6 copies/mL.

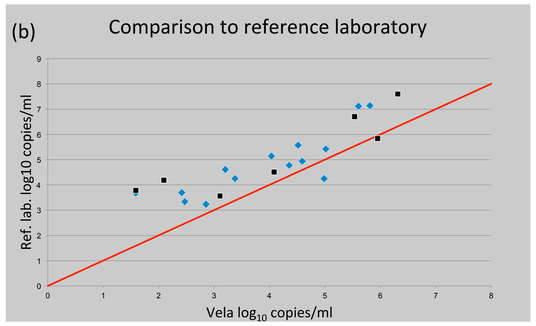
Precision: Precision in plasma was analyzed using high and low positive controls (Vela Diagnostics). High and low positive urine controls were generated by diluting a positive sample into pooled negative urine. Each control was tested over 11 days for a total of 22 replicates per control.

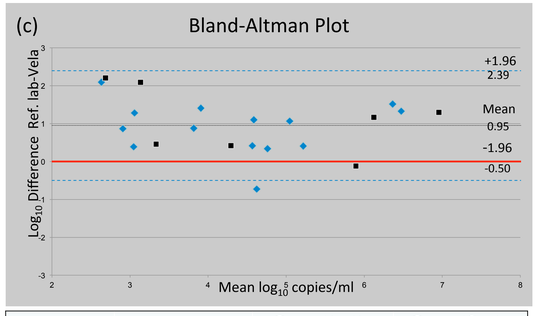
Accuracy: Results for the last 8 CAP proficiency samples were compared to the results reported in the participants summary. In addition, 78 samples were tested in parallel with our current send out reference laboratory.

Results

Accuracy



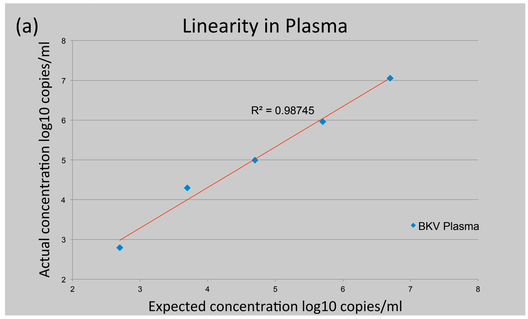


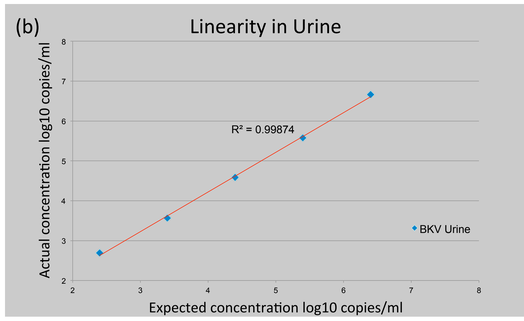


(d)	Accuracy	Sensitivity	Specificity
Total	87.18%	92.00%	84.91%
Plasma	83.64%	88.24%	81.58%
Urine	95.65%	100.00%	93.33%

Accuracy: (a) Results for the last 8 CAP proficiency samples were compared to the median and range of CAP results reported in the participants summary; the Vela BKV results were on average 0.7$\log_{10}$ copies/mL higher; **(b)** Sentosa results for 78 samples (55 plasma (blue diamond) and 23 urine (black square)) were compared to the results of our current reference laboratory; **(c)** Bland-Altman plot showed 1/20 results to fall outside 95% confidence limits and **(d)** the qualitative accuracy of the assay was 87.2% overall.

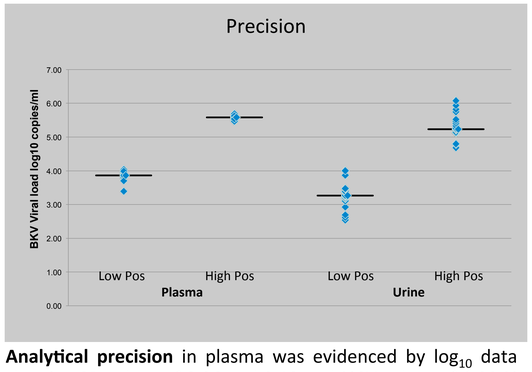
Linearity





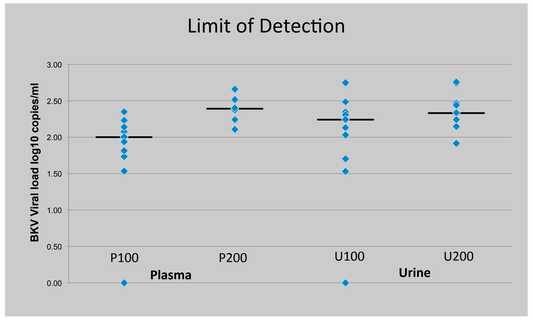
Reportable Range (linearity) was determined using an AcroMetrix panel of 5 BKV concentrations. The assay generated linear quantitative results (R^2=0.9875 and R^2=0.9987) between **(a)** 5x10^2 to 5x10^6 copies/mL (EDTA plasma) and **(b)** 2.5x10^2 to 2.5x10^6 copies/mL (Urine).

Precision



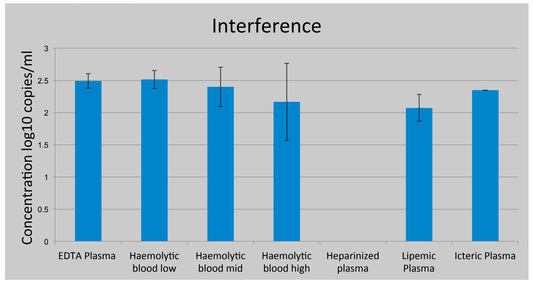
Analytical precision in plasma was evidenced by $\log_{10}$ data showing SD values of 0.06 and 0.13 and %CV values of 1.00 % and 3.38% for positive high control and positive low control respectively. The standard deviation values for urine were 0.36 and 0.35 and %CV values of 6.84 % and 10.66% for positive high control and positive low control respectively.

Analytical sensitivity



Analytical Sensitivity (limit of detection, LOD): To determine LOD a previously tested positive sample was diluted into negative plasma and negative urine for calculated concentrations of 200 copies/mL ($\log_{10}$=2.30) (P200 and U200) and 100 copies/mL ($\log_{10}$=2.00) (P100 and U100). Aliquots were tested over 5 days. All samples were detected at the higher concentration and 9/10 (90%) of the aliquots at the lower concentration generated a positive result. The package insert indicates that the LOD is 187 copies/ml.

Interference



Interference was addressed using using an AcroMetrix Inhibition panel designed to mimic potential interfering substances and clinical conditions, such as hemolysis, icterus, and lipemia. Experiments to address inhibition in urine and cross reactivity is currently in progress. The Vela BKV assay incorporates an external extraction/inhibition control.

Conclusions

The results validate the reportable range, accuracy, precision and sensitivity of the Sentosa BKV quantitative assay for BK virus. Its wider adoption might alleviate problems with inter-laboratory variability associated with the multitude of laboratory developed tests that are currently in use.

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