# Utility of droplet digital PCR for the quantitative detection of BK polyomavirus in blood from kidney transplant recipients

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## Introduction

BKV reactivation in kidney transplant recipients is asymptomatic and can progress to polyomavirus-associated nephropathy (PVAN) and allograft loss (1-3). Quantitative real-time PCR (qPCR) is recommended for detection and monitoring of BKV viremia, despite limitations in accuracy and standardisation of results due to intra- and inter-laboratory variability and BKV subtype-dependent quantification bias (2-5).

Droplet digital PCR (ddPCR) provides absolute quantification of target DNA without the need for external standard curves, and thus offers potential advantages for BKV testing.

## Aim

Our aim was to compare ddPCR to a reference BKV qPCR for the quantitative detection of BKV in blood samples obtained from kidney transplant recipients.

## Methods

Plasma samples submitted for BKV qPCR between September 2014 and May 2018 were tested at a referral laboratory using an in-house quantitative real-time TaqMan® assay. Six specific primers and probes previously published in the literature were manufactured by Sigma-Aldrich Pty Ltd (Castle Hill, NSW) and initially analysed qualitatively by qPCR (Roche LightCycler®). Absolute quantification of BKV DNA in clinical samples was then performed by ddPCR (Bio-Rad QX200 AutoDG™ Droplet Digital PCR system) using the best performing assay and compared to results obtained using the reference method.

## Results

Twenty-eight EDTA plasma samples previously tested with the reference method were analysed using ddPCR (Table 1). The overall categorical agreement between assays was 96.4% (27/28) with a kappa statistic of 0.9 (95% CI, 0.82 to 0.99). There was only one discrepant sample, which was positive by the reference method and negative by BKV ddPCR. This sample had a low viral load of only 18 copies/mL by the reference method.

| BKV ddPCR | BKV qPCR | | |
|---|---|---|---|
| | Detected | Not detected | Total |
| Detected | 21 | 0 | 21 |
| Not detected | 1 | 6 | 7 |
| Total | 22 | 6 | 28 |

***TABLE 1*** *Comparison of the BKV qPCR reference method and BKV ddPCR assays for detection of BKV DNA in clinical samples.*

The analytical sensitivity was 95.5% (95% CI, 77.2% to 99.9%) and the specificity was 100.0% (95% CI, 54.1% to 100.0%). The Youden index was 0.95 [(0.95+1.0)-1 = 0.95] indicating near perfect diagnostic accuracy compared with the reference method.

The 21 clinical samples which were quantifiable by both methods were analysed for quantitative agreement. Following logarithmic transformation, the extent of viral load agreement in log10 copies/mL was examined by plotting the differences between the pairs of measurements on the vertical axis, against the mean of each pair on the horizontal axis in a Bland-Altman plot. There was a systematic tendency for viral loads measured by BKV qPCR to be lower than those measured by BKV ddPCR by an average of 0.53 log10 copies/mL (95% CI -0.81 to -0.27) indicating that the methods were not in sufficient agreement (Figure 1).

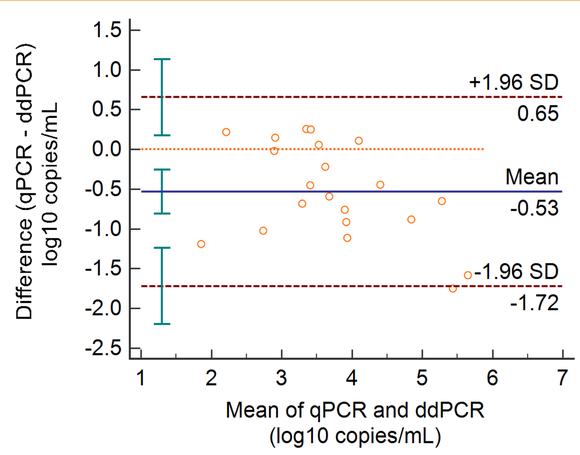


***FIGURE 1*** *Bland-Altman plot of the differences between the pairs of measurements on the vertical axis, against the mean of each pair on the horizontal axis. The solid blue horizontal line corresponds to the mean difference (-0.53 log10 copies/mL) while the dotted red horizontal lines correspond to the 95% limits of agreement.*

## Conclusions

Viral loads measured by ddPCR were higher than those measured by the reference method. Droplet digital PCR is less affected by sequence variation, PCR efficiency bias and PCR inhibitors, and therefore it is possible that the viral loads obtained by the reference method were an underestimate of the true value.

ddPCR may have advantages over qPCR in monitoring for BKV viremia in patients at risk of PVAN, however, further evaluation is required in prospective studies comparing viral load values with clinical outcomes and the results of histopathology where available.

## References

1. Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al. Manual of Clinical Microbiology, Eleventh Edition: American Society of Microbiology; 2015.
2. Ambalathingal GR, Francis RS, Smyth MJ, Smith C, Khanna R. BK Polyomavirus: Clinical Aspects, Immune Regulation, and Emerging Therapies. Clinical microbiology reviews. 2017;30(2):503-28.
3. Hirsch HH, Randhawa P. BK polyomavirus in solid organ transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2013;13 Suppl 4:179-88.
4. Sawinski D, Goral S. BK virus infection: an update on diagnosis and treatment. Nephrol Dial Transplant. 2015;30(2):209-17.
5. DIGO clinical practice guideline for the care of kidney transplant recipients. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2009;9 Suppl 3:S1-155.

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