Quantitative Detection of BK Virus in Whole Blood, Plasma, and Urine Using a Commercial Sample-to-Answer, Automated System

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Abstract*

Background: A simple, automated solution to quantify BKV across a wide linear range with high sensitivity, specificity, and quantitative accuracy is needed. Automated systems offer the potential to reduce time to result and improve operational efficiency by providing: an individualized, automated solution enabling personalized preparation and detection. The ARIES® automated PCR system (Luminex Corporation, Austin, TX) has been used primarily for qualitative detection of infectious pathogens. This study was carried out to assess those capabilities and the potential for a better approach against a laboratory developed-real-time quantitative PCR assay (LDT).

Introduction

The potential of such an approach offers multiple instruments and hours of hands-on time. The recent availability of commercial sample-to-answer PCR systems offers the potential to reduce time to result and improve operational efficiency by providing: an individualized, automated solution enabling personalized preparation and detection. The ARIES® automated PCR system (Luminex Corporation, Austin, TX) has been used primarily for qualitative detection of infectious pathogens. Here we evaluate its use for the quantitative detection of BKV (in three different sample matrices) and compared a clinical and analytical performance against a laboratory developed-real-time quantitative PCR assay (LDT).

Materials/Methods: Quantitative standards in plasma matrix (2.3 – 3.0 log10 IU/mL) were purchased (Exact Diagnostics, Fort Worth, TX) and seeded into BKV negative whole blood (WB), and urine to create concentration gradients from 2.3 – 3.7 log10 IU/mL, in all sample types. Samples were loaded into extraction vials and processed on the ARIES® automated system, using MultiCode® BK and Control Primers (Luminex). Results were analyzed using the LinX™ Software-package. Limits of quantitation and linear range, between-and-within instrument (instrument position) variability were tested. Eighty-four deidentified blood and 79 urine patient samples were tested (both BVV positive and negative). The results were compared to several of the "conventional" real-time PCR methods.

Results: Linearity was seen across all concentrations tested (2.3 – 3.7 log10 IU/mL) in plasma and WB. Multiple BVV primers (2 µL) and MHV control primers (2 µL) (Luminex Corporation, Austin, TX) were added to the DNA Ready-Mix tube (Luminex) and then attached to the microsphere surface. Amplification and detection all occurred within the instrument. Results were analyzed using the LinX™ software package (Luminex).

Discussion: A total of 59 de-identified clinical blood and 79 clinical urine samples were tested to assess sensitivity, specificity, and quantitative accuracy. Results were correlated with those from currently utilized laboratory developed test (LDT) targeting BKV, utilizing real-time quantitative PCR on an ABI 7500 Thermocycler (Thermofisher Scientific, Waltham, MA). Linear regression was used to determine the correlation between the two methods. The sensitivity, specificity, and accuracy were determined at different sample concentrations. Sensitivity and specificity were estimated, along with 95% confidence intervals, using OLS as the reference standard.

Conclusions

Automated, sample-to-answer PCR can be used to quantify BKV across a wide linear range with a high degree of reproducibility, in both blood and urine samples. Quantitative accuracy was similar to that of the evaluated LDT, and reduced specificity may represent false negativity by the reference method. Automated systems offer potential savings in time and labor for the clinical laboratory. These results support further evaluation of such methodology is warranted.

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Note: St. Jude Children’s Research Hospital does not endorse any tests or other products of the Luminex Corporation by allowing them to make this poster available.

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Figure 1. BK virus linear range across seven concentrations for three matrices

Table 1. Results

Figure 2. Lower limit of quantification, ARIES®

Table 2. Coefficient of determination for each replicate varied between 0.01 and 0.98; the % of the mean of replicates values was 0.98. Table 3 and Figure 2. The 95% LOD was determined to be 4,913 copies/mL (3.7 log10 copies/mL).

Table 3. Twenty-four replicates were run in all concentrations to estimate the LOD. The highest level of result variability was seen at 3.0 log10 cp/mL, 3.6 level of quantification (LOD) and detection (LOD). A concentration range of 3.0–3.2 log10 cp/mL was tested using multiple sets of m/z 595 (n=4 replicates per concentration level), to estimate LOD. Each matrix, fifteen replicates, five in three experimental runs, were tested to determine the LOD. The greatest level of variability is between high and among samples. Precision was assessed by estimating the coefficient of variation (CV) within and among replicates, linear regression was used to assess quantitative range, and the linear regression was used to determine variability. The 95% LOD was determined to be 4,913 copies/mL (3.7 log10 copies/mL). The LOD patient sample.

Figure 3. Accuracy of ARIES® in blood and urine patient samples

Figure 4. Accuracy of ARIES® in blood and urine patient samples

A. Accuracy of ARIES® in detecting BKV in clinical blood samples
B. Accuracy of ARIES® in detecting BKV in clinical urine samples
