Performance Characteristics of a Fully Automated, Random-Access qPCR Assay for Monitoring BKV DNA in Plasma and Urine Specimens

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Background

BK virus (BKV) infection is linked to two major complications: BKV associated nephropathy (BKVAN) in kidney transplant patients and BKV associated hemorrhagic cystitis in hematopoietic stem cell transplant patients. In the absence of BKV specific therapy and limited treatment options for advanced BKVAN, active screening of BKV replication in urine and blood combined with pre-emptive modulation of immunosuppression therapy are essential measures to prevent BKVAN. PCR-based viral load quantitation in plasma and urine is the standard clinical tool for monitoring BKV reactivation and response to treatment. Studies reporting quantitative BKV PCR results demonstrate a positive correlation between higher viral loads and an increased probability of developing BKVAN. The NeuMoDx® BKV Quant Assay enables rapid quantitative and specific detection of BKV in urine and plasma matrices.

Material and methods

The NeuMoDx BKV Quant Assay combines automated DNA extraction, amplification and detection by real-time PCR. Performance of the NeuMoDx BKV Quant Assay was characterized in both urine and plasma using a specimen input volume of 550 µl. The performance of the NeuMoDx BKV Quant Assay was demonstrated across key analytical metrics including analytical sensitivity (LoD), linearity, precision, turnaround time and specificity. Evaluation of analytical sensitivity was performed using the 1st WHO International Standard for BKV, and the limits of quantitation (LLoQ/ULoQ) were determined using the TAE ≤1.0 criterion. The study was performed over 3 days across multiple systems with multiple lots of NeuMoDx reagents. Each system processed 42 replicates at each dilution level (positive samples) and 8 replicates for negative samples per day.

Analytical sensitivity

LoD

Analytical sensitivity was characterized by testing a dilution series of the EDX BKV Verification Panel, calibrated against the 1st WHO International Standard in BKV, to determine the LoD on the NeuMoDx Systems. For plasma/serum and urine, LoD was defined as the closest target level, above the concentration determined by Probit-style analysis with 95% CI. The study was performed over 3 days across multiple systems with multiple lots of NeuMoDx reagents.

Positive detection rates for LoD determination of the NeuMoDx BKV Quant Assay

Target conc. (IU/ml)	Target conc. (Log_{10} IU/ml)	Plasma/serum			Urine		
		No. valid tests	No. positives	Detection rate	No. valid tests	No. positives	Detection rate
50	1.70	41	41	100%	41	41	100%
20	1.30	42	42	100%	40	39	98%
10	1.00	41	35	85%	41	31	76%
5	0.30	41	16	39%	41	12	29%
NEG	0.00	20	0	0%	24	0	0%

The LoD of the NeuMoDx BKV Quant Assay in plasma/serum (550 µl workflow) was 20 IU/ml (1.3 Log_{10} IU/ml) with 95% CI: 11.03. In urine, the LoD was 20.0 IU/ml (1.3 Log_{10} IU/ml) with 95% CI: 13.09.

LLoQ and ULoQ

Lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ) are defined as the upper target levels at which >95% detection is achieved AND the total analytical error* (TAE) ≤1.0. To determine LLoQ and ULoQ, the TAE was calculated for each of the BKV target levels >95% detection.

*TAE: Bias + 2*SD (Westgard Statistic)

NeuMoDx BKV Quant Assay ULoQ and LLoQ, with bias and TAE

Target conc. (IU/ml)	Target conc. (Log_{10} IU/ml)	Plasma/serum					Urine				
		Average conc. (Log_{10} IU/ml)	Detection (%)	SD	Bias	TAE	Average conc. (Log_{10} IU/ml)	Detection (%)	SD	Bias	TAE
$7.58x10^7$	8	8	100	0.09	0.05	0.23	8	100	0.09	0.10	0.29
50	1.70	1.80	100	0.18	0.10	0.46	1.84	100	0.22	0.14	0.59
20	1.30	1.56	100	0.25	0.26	0.76	1.66	100	0.29	0.36	0.93
10	1.00	1.46	85	0.27	0.46	1.01	1.41	76	0.41	0.41	1.22
5	0.30	1.27	39	0.48	0.97	1.92	1.31	29	0.52	1.01	2.04

Based on this data set and the determined LoD, the LLoQ and ULoQ were determined to be 20 IU/ml (1.3 Log_{10} IU./ml) and 7.58 x 107 IU/ml (here approximated to 8 Log_{10} IU/ml), respectively for plasma/serum 550 µl and urine.

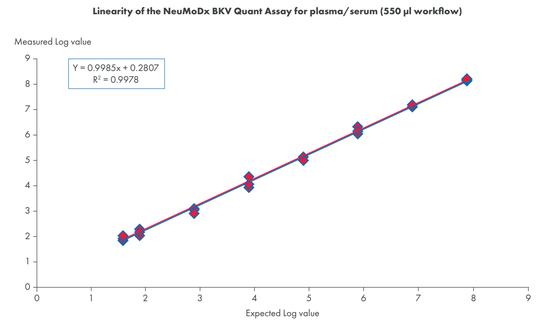
Analytical sensitivity: linearity

Linearity was established in plasma/serum and urine by preparing a dilution series using BKV synthetic plasmid with established traceability to the 1st WHO International Standard for BK virus. 11 serial dilutions, prepared in BKV-negative Base Matrix 53 or pooled BKV-negative human urine, were created to span a concentration range of 8–1.58 Log_{10} IU/ml for plasma/serum 550 µl and urine.

Linearity of three BKV genotypes (BK Virus Dunlop, BK Virus Gardner, BK Virus AB269822_FIN-2) was characterized by testing four different concentrations of each genotype of BKV. Linearity across three BKV genotypes is presented in the table.

Linearity of the NeuMoDx BKV Quant Assay across genotypes

Genotype	Linearity equation y = NeuMoDx BKV Assay C_t, x = Dilution series	R^2
BK Virus Dunlop	y = –3.4808x + 0.8595	0.9926
BK Virus Gardner	y = –3.4682x + 0.6395	0.9959
BK Virus AB269822_FIN-2	y = –3.432x + 1.2683	0.9947



Linearity of the NeuMoDx BKV Quant Assay for plasma/serum (550 µl workflow).

Analytical specificity: cross-reactivity and interfering substances

Cross-reactivity: Analytical specificity was demonstrated by screening 22 organisms commonly found in plasma/serum or urine specimens, as well as species phylogenetically similar to BKV for cross-reactivity. No cross-reactivity was observed with any of the organisms tested, confirming 100% analytical specificity of the NeuMoDx BKV Quant Assay.

Interference testing – exogenous and endogenous agents

Endogenous (plasma/serum)	Log_{10} average conc. Log_{10} IU/ml	Bias Log_{10} IU/ml
Triglycerides 500 mg/dl	3.09	0.16
Conjugated bilirubin (0.25 g/l)	3.09	0.16
Unconjugated bilirubin (0.25 g/l)	3.31	–0.06
Albumin (58.7 g/l)	3.12	0.13
Hemoglobin (2.9 g/l)	3.02	0.23

Endogenous (urine)	Log_{10} average conc. Log_{10} IU/ml	Bias Log_{10} IU/ml
Urobilirubin (> 2 mg/dl)	3.74	–0.14
Glucose (1000 mg/dl)	4.00	0.04
Urine pH 4	3.75	0.29
Urine pH 10	3.77	0.27
Leucocytes (1E5 cells/ml)	3.68	–0.20
Blood 7%	4.42	–0.46
Protein (albumin >100 mg/dl)	3.96	0.08
Talcum powder	3.92	0.12

Exogenous (drugs)	Log_{10} average conc. Log_{10} IU/ml	Bias Log_{10} IU/ml
Pool 1: Valganciclovir, prednisone, cidofovir, cefotaxime, mycophenolate mofetil	4.04	–0.06
Pool 2: Vancomycin, tacrolimus, famotidine, valacyclovir, leflunomide	4.07	–0.09

Interfering substances: The NeuMoDx BKV Quant Assay was evaluated in the presence of typical exogenous and endogenous interfering substances encountered in BKV clinical plasma/serum or urine specimens. Each substance was spiked with 3 Log_{10} IU/ml BKV and samples were analyzed for interference. None of the exogenous and endogenous substances affected the specificity of the NeuMoDx BKV Quant Assay.

Precision and reproducibility

Within-lab precision

Determined using a 5-member panel of BKV prepared with BKV plasmid to assess performance on one NeuMoDx 96 Molecular System across three separate runs. Maximum overall bias was 0.27 Log_{10} IU/ml. Equivalent performance was demonstrated across lots as quantitation of all panel members was within tolerance specification.

Lot-to-lot reproducibility

Precision was determined by testing two replicates of a 5-member panel of BKV specimens prepared with BKV plasmid twice daily for 20 days. Excellent precision was demonstrated across days and runs.

Instrument-to-instrument reproducibility

Determined using three different systems (two NeuMoDx 288 Molecular System and one NeuMoDx 96 Molecular System). A 5-member panel of BKV prepared with BKV plasmid was used to assess performance. Testing was performed in parallel on the systems for 5 days. The variation within-day and between systems was characterized, and the overall standard deviation was determined to be ≤0.30 Log_{10} IU/ml. Equivalent performance was demonstrated across systems as SD in quantitation of all panel members was within tolerance specification.

Within Lab Precision – NeuMoDx BKV Quant Assay on NeuMoDx Systems

Sample	Within-day SD (Log_{10} IU/ml)	Between-day SD (Log_{10} IU/ml)	Within-run SD (Log_{10} IU/ml)	Between-run SD (Log_{10} IU/ml)	Within-lab SD (Log_{10} IU/ml)
Plasma/ serum specimen (input 550 µl)					
RISppHIGH	0.10	0.08	0.10	0.01	0.13
RISppMIDDLE	0.14	0.10	0.11	0.06	0.17
RISppMLOW	0.22	0.12	0.21	0.02	0.25
RISppLOW	0.21	0.03	0.18	0.10	0.21
RISppNEG	0.00	0.00	0.00	0.00	0.00
Urine specimen (550 µl)					
RISpuHIGH	0.16	0.10	0.11	0.12	0.20
RISpuMIDDLE	0.21	0.09	0.16	0.13	0.23
RISpuMLOW	0.14	0.12	0.13	0.02	0.18
RISpuLOW	0.29	0.05	0.25	0.13	0.30
RISpuNEG	0.00	0.00	0.00	0.00	0.00

Lot to Lot Reproducibility – NeuMoDx BKV Quant Assay

Sample	Absolute bias between Lot 1 and Lot 2 (Log_{10} IU/ml)	Absolute bias between Lot 1 and Lot 3 (Log_{10} IU/ml)	Absolute bias between Lot 2 and Lot 3 (Log_{10} IU/ml)
Plasma/ Serum specimen (550 µl)			
7 Log_{10} IU/ml	0.05	0.10	0.05
4 Log_{10} IU/ml	0.02	0.03	0.05
3 Log_{10} IU/ml	0.20	0.05	0.15
2 Log_{10} IU/ml	0.02	0.24	0.26
0 Log_{10} IU/ml	0.00	0.00	0.00
Urine specimen (550 µl)			
7 Log_{10} IU/ml	0.09	0.27	0.19
4 Log_{10} IU/ml	0.25	0.06	0.19
3 Log_{10} IU/ml	0.06	0.08	0.14
2 Log_{10} IU/ml	0.03	0.11	0.14
0 Log_{10} IU/ml	0.00	0.00	0.00

Instrument to instrument Reproducibility – NeuMoDx BKV Quant Assay

Sample	Repeatability SD (Log_{10} IU/ml)	Between-day SD (Log_{10} IU/ml)	Within-instrument SD (Log_{10} IU/ml)	Between-instrument (Log_{10} IU/ml)	Reproducibility SD (Log_{10} IU/ml)
Plasma/ serum specimen (input 550 µl)					
RISppHIGH	0.10	0.05	0.11	0.06	0.12
RISppMIDDLE	0.13	0.05	0.13	0.04	0.13
RISppMLOW	0.10	0.06	0.12	0.04	0.12
RISppLOW	0.12	0.05	0.13	0.04	0.13
RISppNEG	0.00	0.00	0.00	0.00	0.00
Urine specimen (550 µl)					
RISpuHIGH	0.11	0.05	0.12	0.06	0.14
RISpuMIDDLE	0.10	0.01	0.10	0.05	0.11
RISpuMLOW	0.09	0.04	0.10	0.07	0.12
RISpuLOW	0.15	0.02	0.15	0.05	0.16
RISpuNEG	0.00	0.00	0.00	0.00	0.00

Conclusion

The NeuMoDx BKV Quant Assay provides a rapid, easy-to-use and automated method for detection and quantification of BKV DNA in plasma and urine specimens.

The NeuMoDx BKV Quant Assay is for in vitro diagnostic use.

Sample to Insight