Validation of BK virus R-gene™ assay on iQ5 Real-Time PCR Detection System

L. Claeys[1], B. Kabamba[2], P. Goubau[2] and E. Padalko[1]

[1]Clinical Biology Laboratory, Ghent University Hospital, Ghent, Belgium

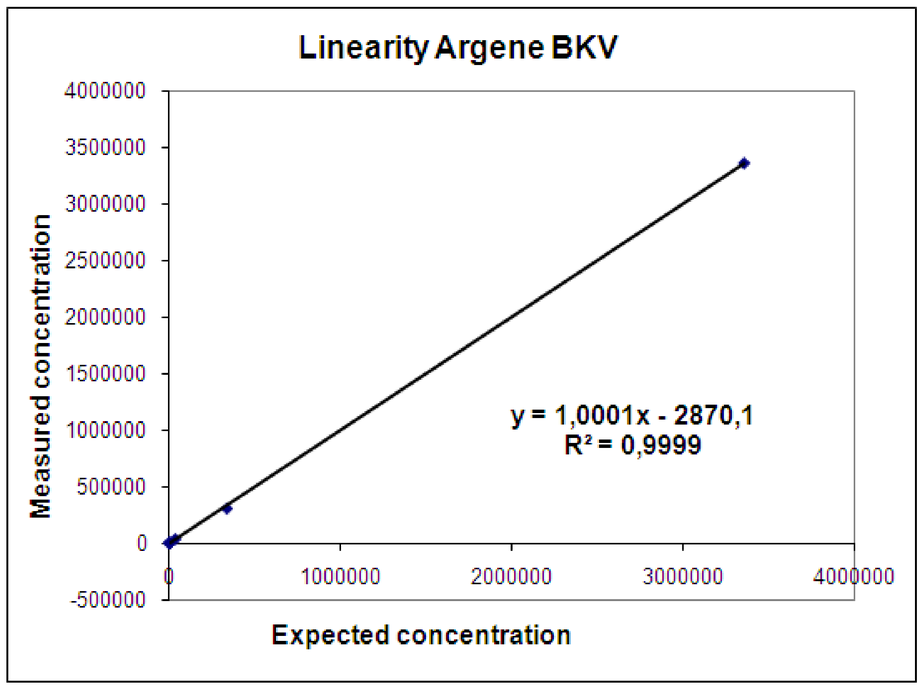
[2]Microbiology Unit, Université Catholique de Louvain, Brussels, Belgium

OBJECTIVES:

BKV may cause nephropathy in renal transplant recipients receiving immunosuppressive therapy, resulting in renal dysfunction and, possibly, graft loss. According to KDIGO clinical practise guideline reduction in immunosuppressive's dose should be accomplished if BKV in plasma is repetitively >10 000 copies/ml. The aim of this work was to validate the use of BK virus R-geneTM quantification assay (Argene) on iQ5 Real-Time PCR Detection System (Biorad).

PATIENTS and METHODS:

Method trueness was validated using the 2010 QCMD JC&BK virus EQA panel, comparing observed with expected results. 0.5 log was the maximum difference accepted.
Between-run and within-run imprecision were determined on repetitive detection of high and low positive samples.
Detection limit was calculated by probit analysis using replicates of dilutions of standards.
Linearity was calculated using dilutions of a high positive patient's sample.
Cross reactivity was investigated using EQA samples positive for viruses genetically or clinically related to BKV (EBV, CMV, Adenovirus, HPV, JCV).
Finally, 21 positive and 9 negative clinical samples were compared to an external method making no differentiation between BKV and JCV viruses. Results below clinical cut-off (10000 copies/ml) for one method had to be below cut-off using the other method and vice versa. In case of discrepancies, a third method (in-house PCR for BKV) was performed in Université Catholique de Louvain and JCV was detected by JC virus R-gene™ (Argene).



RESULTS:

All QCMD-samples were identified and quantified correctly. Within-run CV's for the high and low positive samples were 0.25% and 1.09% respectively. Between-run CV's for the high and low positive samples were 2.75% and 3.85% respectively. Limit of detection was 136 copies/ml, compared to a detection limit of 65 copies/ml stated in the kit manual. The linearity experiment resulted in a perfectly linear curve (R^2=0.9999). No cross reactivity was observed.
Method comparison gave equal results for negative samples. For the positive samples, 8/21 (38%) gave discrepant results in relation to clinical cut-off. All but one of the discrepant results were confirmed by third method as concordant with BK virus R-gene™ results. The positivity for JCV was found in 4/8 (50%) of discrepant samples.

CONCORDANT POSITIVE SAMPLES

SAMPLE N°	KUL BKV (copies/ml)	KUL BKV (log copies/ml)	UZ Gent BKV (copies/ml)	UZ Gent BKV (log copies/ml)
1	272134	5,43	21100	4,32
2	107413	5,03	10900	4,04
3	93099	4,97	16000	4,20
4	1187	3,07	110	2,04
5	958	2,98	27,6	1,44
6	6149	3,79	27,8	1,44
7	541	2,73	< dl	/
8	1959	3,29	119	2,08
9	< 500	/	<dl	/
10	< 500	/	4,34	0,64
11	< 500	/	64	1,81
12	< 500	/	21,3	1,33
13	< 500	/	105	2,02

DISCORDANT POSITIVE SAMPLES

SAMPLE N°	method 1 (copies/ml)	method 1 (log copies/ml)	UZ Gent BKV (copies/ml)	UZ Gent BKV (log copies/ml)	method 3 (copies/ml)	method 3 (log copies/ml)	JCV (Ct)
1	17975	4,25	< dl	/	<dl	/	33,14
2	45539	4,66	< dl	/	<dl	/	32,34
3	48915	4,69	< dl	/	<dl	/	32,72
4	12571	4,10	< dl	/	<dl	/	35,33
5	641879	5,81	< dl	/	<dl	/	0,00
6	48915	4,69	6390	3,81	2402	3,38	0,00
7	4962	3,70	46100	4,66	11568	4,06	0,00
8	1823	3,26	10800000	7,03	<dl	/	0,00

CONCLUSION:

The BK Virus R- gene™ assay shows performant characteristics if performed on the iQ5 Real-Time PCR Detection System. One should consider the specificity of the BKV assay, especially regarding simultaneous detection of JCV.