A rapid genotyping method for Polyomavirus BK

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Introduction

Polyomavirus BK (BKV) can cause nephropathy in renal transplant recipients or hemorrhagic cystitis in bone marrow recipients. BKV can be classified in 4 main genotypes I-IV, which differs in prevalence and distribution. Until now it is not clear whether there is a difference among the various BKV genotypes and their causative role in development of nephropathy and hemorrhagic cystitis. The availability of a rapid, simple and cost-effective genotyping tool could be useful for studies, which investigate the impact of the role of BKV infection in relation to the genotype.

Methods

- A multiplex of Real Time PCRs (RT-PCR) was developed and validated on the VP1 gene to differentiate the 4 main genotypes of BKV.
- The BKV RT-PCR is on the VP2 gene.
- 150 BKV positive samples (17 plasma, 133 urine) were tested with these specific assays.
- Of these 150 samples, 50 were additionally confirmed by sequencing the 1630-1956 VP1 nucleotide fragment.

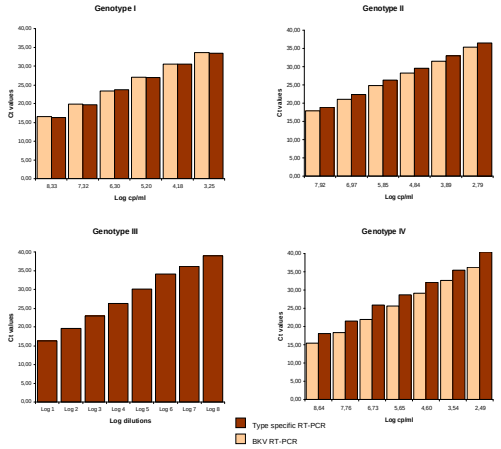
Results

Table 1 Precision (intra-inter assay variation)

The intra-inter assay variation was assessed by testing log-fold serial dilutions in triplicate. For genotypes I, II en IV patient material were used and for genotype III a plasmid.

		Intra-assay				Inter-assay			
BKV Subtype	Dilution	(Average Ct ± SD (%CV))				(Average Ct ± SD (%CV))			
BK GT1	Undiluted	19,74	±	0,09	(0,44)	19,50	±	0,18	(0,92)
	Log 1	23,29	±	0,06	(0,28)	23,70	±	0,33	(1,38)
	Log 2	26,97	±	0,09	(0,32)	26,56	±	0,41	(1,55)
	Log 3	30,27	±	0,16	(0,53)	29,99	±	0,46	(1,53)
	Log 4	34,01	±	0,19	(0,57)	33,35	±	0,65	(1,96)
BK GT2	Undiluted	20,58	±	0,05	(0,23)	20,38	±	0,22	(1,1)
	Log 1	24,15	±	0,05	(0,21)	23,85	±	0,25	(1,07)
	Log 2	28,00	±	0,13	(0,46)	27,58	±	0,41	(1,47)
	Log 3	31,27	±	0,08	(0,25)	31,07	±	0,24	(0,77)
	Log 4	35,15	±	0,06	(0,18)	34,90	±	0,38	(1,09)
BK GT3	Undiluted	18,92	±	0,27	(1,41)	18,52	±	0,5	(2,7)
	Log 1	23,14	±	0,09	(0,41)	23,05	±	0,1	(0,41)
	Log 2	26,37	±	1,18	(4,46)	25,53	±	1,34	(5,25)
	Log 3	30,40	±	0,27	(0,9)	29,74	±	0,43	(1,45)
	Log 4	34,62	±	0,35	(1,01)	33,59	±	1,21	(3,6)
BK GT4	Undiluted	21,54	±	0,12	(0,57)	21,59	±	0,31	(1,45)
	Log 1	25,55	±	0,34	(1,33)	24,98	±	0,41	(1,66)
	Log 2	28,67	±	0,04	(0,15)	28,46	±	0,35	(1,22)
	Log 3	32,05	±	0,32	(0,99)	31,94	±	0,2	(0,63)
	Log 4	36,51	±	0,78	(2,15)	36,03	±	0,38	(1,06)

Fig. 1 Limit of detection BKV Genotypes RT-PCRS



The limit of detections were determined by using log-fold serial dilutions of BKV genotype I t/m IV.
For genotypes I, II and IV patient material was used and for genotype III a plasmid on VP1. All dilutions were tested in duplicate in the BKV RT-PCR.
A specificity of 100% was found in all BKV genotypes RT-PCRs.

Fig. 2 Phylogenetic tree

Maximum parsimony tree of detected BKV strains from UMCG patients (orange dots) and references (blue dots). For phylogenetic analysis, the 1630-1956 VP1 nucleotide fragment was sequenced.
50 samples were tested with the multiplex of type specific RT-PCRs and confirmed by sequencing. In four samples typing was not possible due to a low viral load.

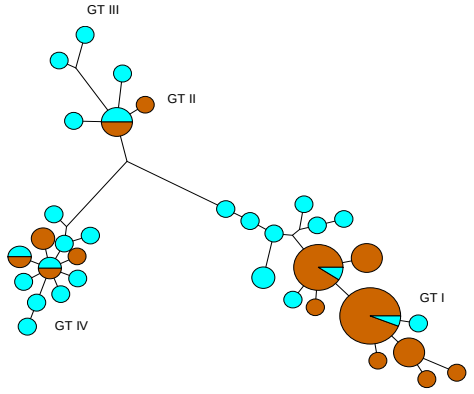
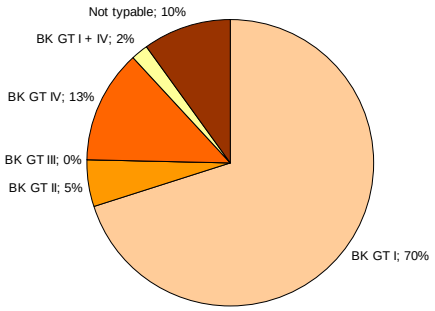


Fig. 3 Different genotypes detected in 150 samples



In total 150 BKV positive samples were tested with the multiplex of type specific RT-PCRs. In 15 samples typing was not possible due to a low viral load (< Log 3 cp/ml).

3 samples had a double infection with genotype I and IV. These samples could also be confirmed by sequencing.

Conclusions

This study describes a new multiplex RT-PCR for detection of the genotypes of BKV.
It proved to be a rapid, cheap and sensitive genotyping tool compared to sequencing,
It can easily detect double infections with different BK genotypes, in contrast to sequencing
This method will be of value to obtain insight in the relation of BKV genotype with nephropathy and hemorrhagic cystitis.

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