

Evaluation of RealStar® BKV PCR and RealStar® JCV PCR Kits for detection of Polyomaviruses in various sample types

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Introduction: Viral load testing by real-time PCR for BK Virus (BKV) and JC Virus (JCV) has become the standard of care for the diagnosis of infection and monitoring of therapy of transplant patients. Laboratory developed tests and commercial assays are used routinely for referring quantitative results. Standardization of quantitation is under development and not yet available (International Standards, WHO).

Objective: This study evaluated the performance of the RealStar® BKV PCR Kit and RealStar® JCV PCR Kit (altona Diagnostics, Germany) for analytical sensitivity, specificity, and correlation in patient samples with laboratory developed real-time PCR tests for BKV and JCV, used by the department of Microbiology, Universitätsklinikum Hamburg-Eppendorf (UKE).

Material & Method I: For initial comparison in qualitative and quantitative detection of Polyomaviruses QCMD 2012 panel sample material for JCV and BKV was tested with both commercial tests (RealStar®, altona Diagnostics) and the lab developed real-time PCR test by UKE (table 1).

Material & Method II: Quantification of viral load was done from eluates of different sample types after automated extraction by NucliSENS® easyMag® (Biomerieux) for retrospective samples (stored at -20° C for up to one year). Prospective routine samples were extracted by QIA Symphony® SP (QIAGEN) using DSP Virus/Pathogen Mini Kit (QIAGEN). All eluates were analyzed in parallel using the RealStar® PCR Kits and lab developed real-time PCR assays on LC480 (Roche).

Results BKV:

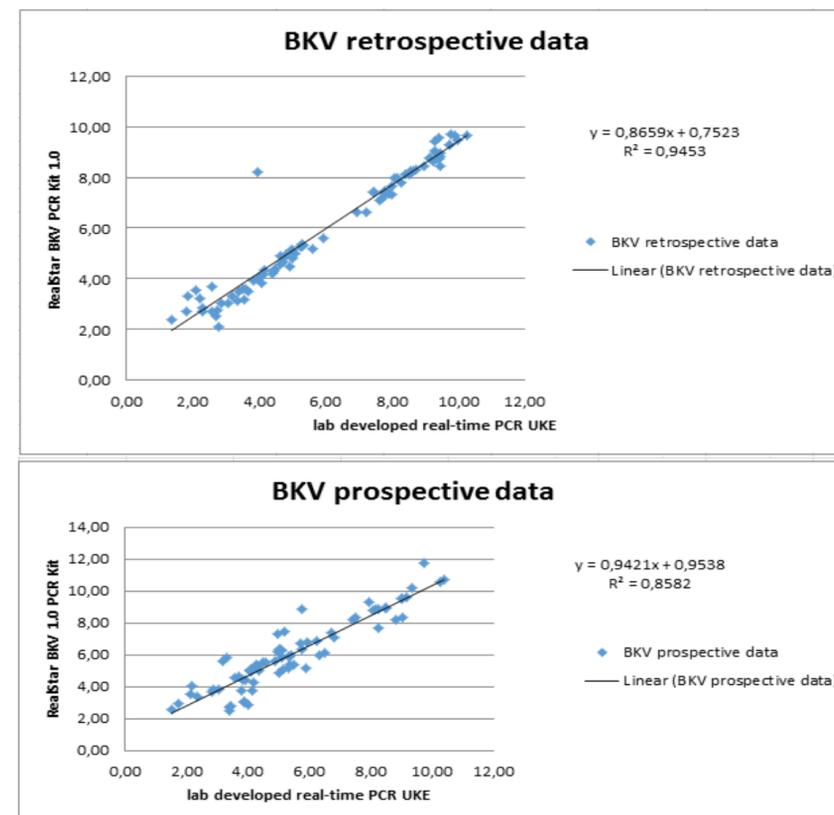


Figure 1: Comparison of quantitative BKV results In total 314 samples were tested for BKV (prospective: 220 samples, retrospective: 94 samples). The majority of samples were urine samples, EDTA blood and serum samples. 137 samples were tested positive and quantified by both assays (lab developed real-time PCR by UKE and RealStar® BKV PCR Kit). All qualitative results are in accordance and no significant differences in viral load can be seen, except in 1 urine sample (>4 log higher in RealStar® BKV PCR). For clarification, this outlier was sequenced and one mismatch were found in the target region of the in-house probe.

Results JCV:

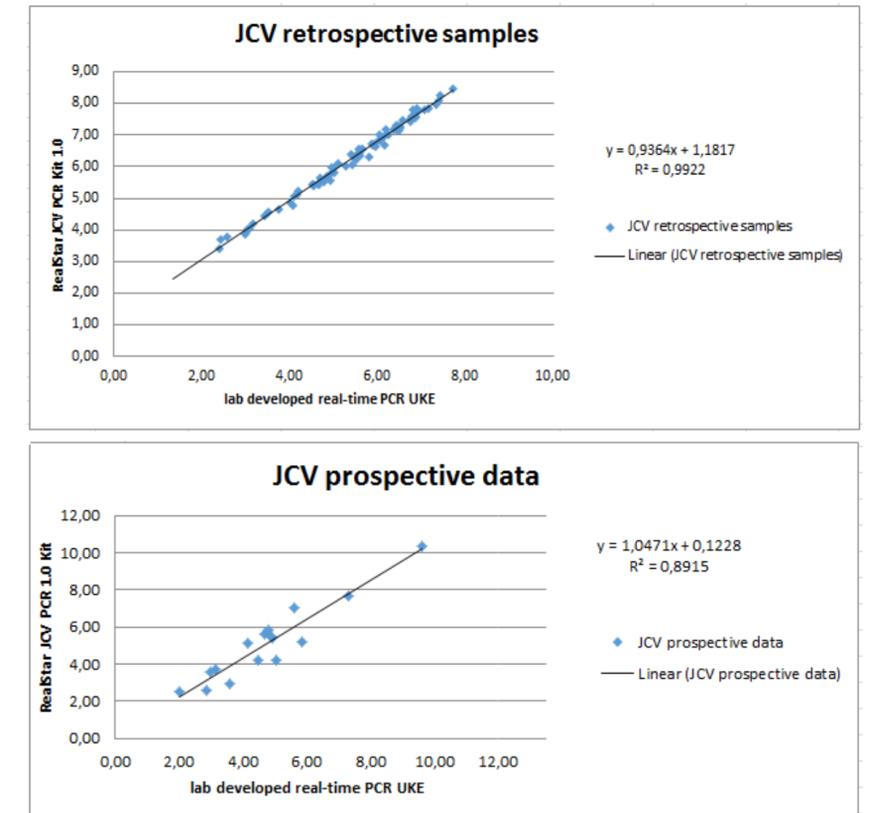


Figure 2: Comparison of quantitative JCV results In total 222 samples were tested for BKV (prospective: 135 samples, retrospective: 87 samples). The majority of samples were urine samples, EDTA blood and serum samples. 98 samples were tested positive and were quantified by both assays (lab developed real-time PCR by UKE and RealStar® JCV PCR Kit). High consistency between both assays can be seen. No significant differences in viral load can be seen.

Conclusion: *The QCMD data indicate a lower sensitivity of the lab developed PCR tests, but analysis of different clinical samples (including serum, urine and liquor) indicate specificity of BKV and JCV by the UKE lab developed and the RealStar® PCR. Furthermore, both assays are highly consistent showing a good linearity of the viral load testing. The BKV outlier in the lab developed test demonstrates that regular updates by sequence checks are mandatory.*

QCMD BK/JC panel 2012

sample	UKE		UKE		altona		altona	
	BK cop/ml	log	JC cop/ml	log	BK cop/ml	log	JC cop/ml	log
1	-		1,29E+02	2,11	-		2,09E+02	2,32
2	<1000*	3	-		4,20E+03	3,62	-	
3	<1000*	3	-		4,70E+02	2,67	-	
4	-		-	0,00	-		3,00E+02	2,48
5	-		-	0,00	-		7,00E+01	1,85
6	-		-		-		-	
7	-	0,00	-		9,00E+01	1,95	-	
8	3,74E+04	4,57	-		5,10E+04	4,71	-	
9	-		1,11E+03	3,05	-		1,50E+03	3,18
10	-		8,14E+02	2,91	-		9,00E+02	2,95
11	-		4,36E+03	3,64	-		1,50E+04	4,18
12	8,10E+04	4,91	-		1,90E+05	5,28	-	

* quantification not accurate
cop/ml below 500

Table 1: QCMD 2012 JC virus and BK virus DNA EQA Program