Evaluation of the QIAsymphony® RGQ system for the quantification of different BK virus genotypes in biological samples

Christel Brunet[2], Gavin Wall[3], Sonia Burrel[1,2], Nathalie Hamm[2], Emmanuel Gay[3], Henri Agut[1,2], David Boutolleau[1,2]

[1]ER1 DETIV, Pierre et Marie Curie University, Paris 6, [2]Virology Department, Pitié-Salpêtrière University Hospital, AP-HP, Paris, France, [3]QIAGEN GmbH, Hilden, Germany

Background and objective

- The ubiquitous BK virus (BKV) belongs to the *Polyomaviridae* family. This virus is classified into 4 major genotypes (I to IV) and 10 additional subgroups within genotypes I and IV **[1]**. BKV constitutes an important pathogen among kidney and haematopoietic stem cell transplant recipients, with related diseases including allograft nephropathy, ureteric stricture and haemorrhagic cystitis. The monitoring of BKV infection in transplant recipients requires the accurate quantification of the viral genome in biological samples using real-time PCR methods. The surveillance of BKV infection, together with the virological surveillance of other opportunistic viral infections in immunocompromised patients, needs high throughput machines able to monitor a large number of biological specimens. The QIAsymphony® RGQ system (QIAGEN) combines the extraction/distribution on the QIAsymphony Sample Preparation (SP) and Assay Setup (AS) modules, together with the amplification step on a Rotor-Gene Q (RGQ) machine.
- The objective of this work was to perform the clinical evaluation of the QIAsymphony® RGQ system for the quantification of BKV DNA in whole blood (tests performed in an off-label capacity) and urine by comparison with the strategy implemented in the laboratory for routine activity: extraction using the MagNA Pure Compact instrument (Roche Diagnostics), followed by an in-house real-time PCR **[2]** performed on the LightCycler® (LC) 480 system (Roche Diagnostics), as previously described **[3]**.

Study design

- BKV loads of 128 biological samples (17 urine and 111 whole blood samples) from immunocompromised patients were compared using both strategies:

QIAsymphony RGQ® system

MagNA Pure Compact / LC 480 combination

- The possible impact of BKV genotype on the viral DNA quantification by the *artus*® BK Virus QS-RGQ Kit was evaluated on the BKV positive samples (BKV genotype was determined using an in-house sequencing technique; see poster **P936**)
- The inter-assay variability of the *artus*® BK Virus QS-RGQ Kit was measured using the quantification standards (QS) 1 to 4

Results

- No PCR inhibition: the internal control (IC) was detected in all samples (IC mean C_T value = 26.70 ± 1.78) apart from 7 urine samples with BKV loads > 7 log
- No cross contamination
- Low inter-assay variability considering the 4 QS: CVs ranging from 1.2 to 2.1% (Figure 1)
- High overall agreement: 96.9% (4 discrepant results with BKV loads < 3.0 log)
- Good correlation for the 113 positive samples in both techniques (Figure 2)
- Low difference of quantification (-0.38 log) between both techniques (Figure 3)
- No significant impact of BKV genotype on viral quantification apart from genotype Ia (Table 1)

Table 1. Impact of BKV genotype on viral quantification

BKV genotype	n	Median BKV load difference (log)
Ia	5	-1.16
Ib1	37	-0.46
Ib2	38	-0.37
II	8	-0.32
III	1	-0.14
IV	24	-0.16

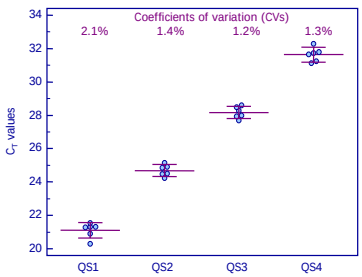

Figure 1. Inter-assay variability

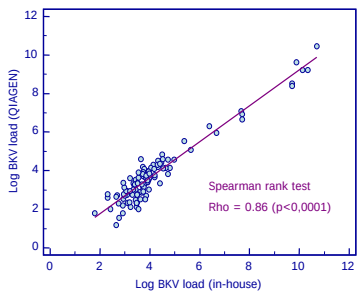

Figure 2. Correlation

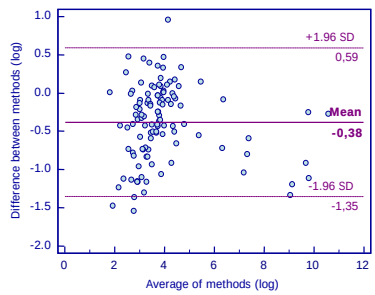

Figure 3. Bland-Altman analysis

Conclusion

- The QIAsymphony® RGQ system is well correlated to the technique implemented in the laboratory for the quantification of BKV load in whole blood and urine samples.
- The potential underquantification of BKV genotype Ia has to be confirmed on a larger panel of samples
- The QIAsymphony® RGQ system offers a fully-automated workflow with improvement in standardisation, traceability and quality control assessment. It appears particularly adapted to the routine surveillance of BKV load in transplant recipients.

References

[1] Luo *et al.*, J Virol 2009; 83: 2285-97.

[2] Hoffman *et al.*, J Clin Microbiol 2008; 46: 2671-80.

[3] Deback *et al.*, J Virol Methods 2009; 159: 291-294.