September 2015

# *artus*® BK Virus QS-RGQ Kit: Performance Characteristics

*artus* BK Virus QS-RGQ Kit, Version 1 REF 4514363

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Sample to Insight

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# Analytical sensitivity – plasma

The analytical detection limit in consideration of the purification (sensitivity limit) was assessed for the *artus* BK Virus QS-RGQ Kit using BK virus-positive clinical specimens in combination with the extraction on the QIAsymphony® SP.

For plasma, the analytical sensitivity in consideration of the purification of the *artus* BK Virus QS-RGQ Kit was determined using a dilution series of BKV material (Acrometrix®) from 316 to nominal 1 BKV copies/ml spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Cellfree1000_DSP protocol (extraction volume: 1 ml, elution volume: 60 µl). Each of the 8 dilutions was analyzed with the *artus* BKV QS-RGQ Kit on 5 different days in 5 runs with 11 replicates each. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Figure 1. The analytical detection limit in consideration of the purification of the *artus* BK Virus QS-RGQ Kit in combination with the Rotor-Gene® Q is 26.67 copies/ml (p = 0.05). This means that there is a 95% probability that 26.67 copies/ml will be detected.

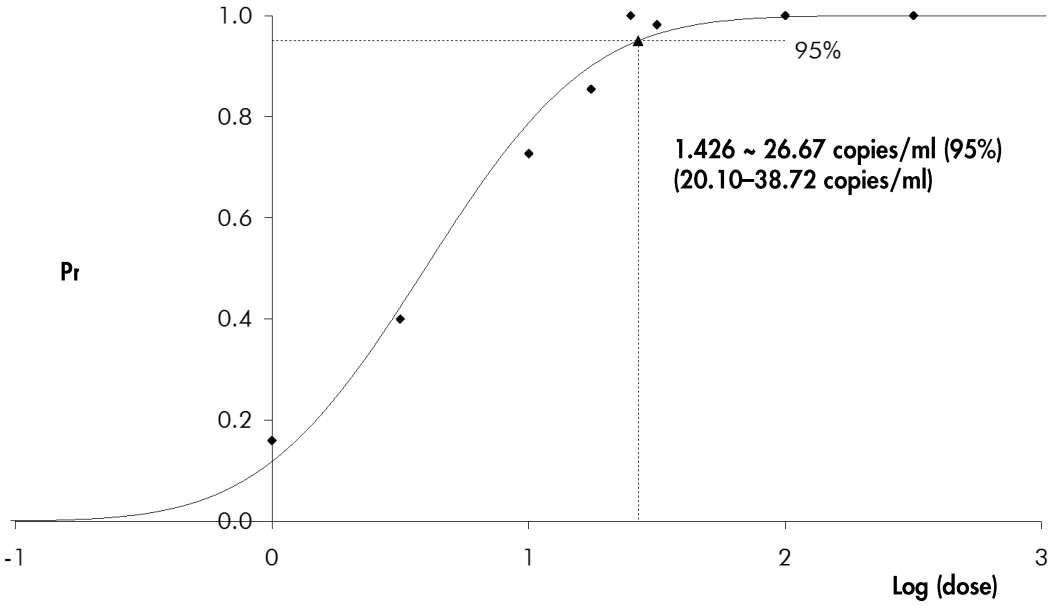


**Figure 1. Probit analysis: plasma, BK virus (Rotor-Gene Q).** Analytical sensitivity in consideration of the purification (plasma, using the QIAsymphony DSP Virus/Pathogen Midi Kit) and the *artus* BK Virus QS-RGQ Kit on Rotor-Gene Q.

# Specificity – plasma

The specificity of the *artus* BK Virus QS-RGQ Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The detectability of all relevant genotypes has thus been ensured by a database alignment and by a PCR run on Rotor-Gene Q instruments with the following genotypes (see Table 1).

**Table 1. Testing the specificity of relevant strains**

| Virus | Strain | Source | BK virus (Cycling Green) | Internal control (Cycling Orange) |
|---|---|---|---|---|
| BK virus | Dunlop | ATCC® | + | + |
| BK virus | Gardner | ATCC | + | + |
| BK virus | AB269822 | Geneart | + | + |
| BK virus | S72390 | Geneart | + | + |

ATCC: American Type Culture Collection.

Moreover, the specificity was validated with 30 different BK virus negative plasma samples. These did not generate any signals with the BK virus specific primers and probes, which are included in the BK Virus RG Master.

A potential cross-reactivity of the *artus* BK Virus QS-RGQ Kit was tested using the control group listed in Table 2. None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

**Table 2. Testing the specificity of the kit with potentially cross-reactive pathogens**

| Control group | BK virus (Cycling Green) | Internal control (Cycling Orange) |
|---|---|---|
| Cytomegalovirus | – | + |
| Epstein-Barr virus | – | + |
| Human herpesvirus 1 (herpes simplex virus 1) | – | + |
| Human herpesvirus 2 (herpes simplex virus 2) | – | + |
| Human herpesvirus 3 (varicella-zoster virus) | – | + |
| Human herpesvirus 6 | – | + |
| JC virus | – | + |
| Simian virus 40 | – | + |
| *Candida albicans* | – | + |

# Linear range – plasma

The linear range in consideration of the purification of the *artus* BK Virus QS-RGQ Kit was determined by analyzing a dilution series of Acrometrix BKV material ranging from $9.26 \times 10^7$ copies/ml to $2.50 \times 10^1$ copies/ml in plasma. The purification was carried out in replicates (n = 4 for concentrations $\geq 1.00 \times 10^7$ copies/ml; n = 8 for concentrations $< 1.00 \times 10^7$ copies/ml) using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Cellfree1000_DSP protocol (extraction volume: 1 ml, elution volume: 60 µl). Each of the samples was analyzed using the *artus* BK Virus QS-RGQ Kit. The linear range in consideration of the purification of the *artus* BK Virus QS-RGQ Kit has been determined to cover concentrations from $5.00 \times 10^1$ copies/ml to $9.26 \times 10^7$ copies/ml for plasma (Figure 2).

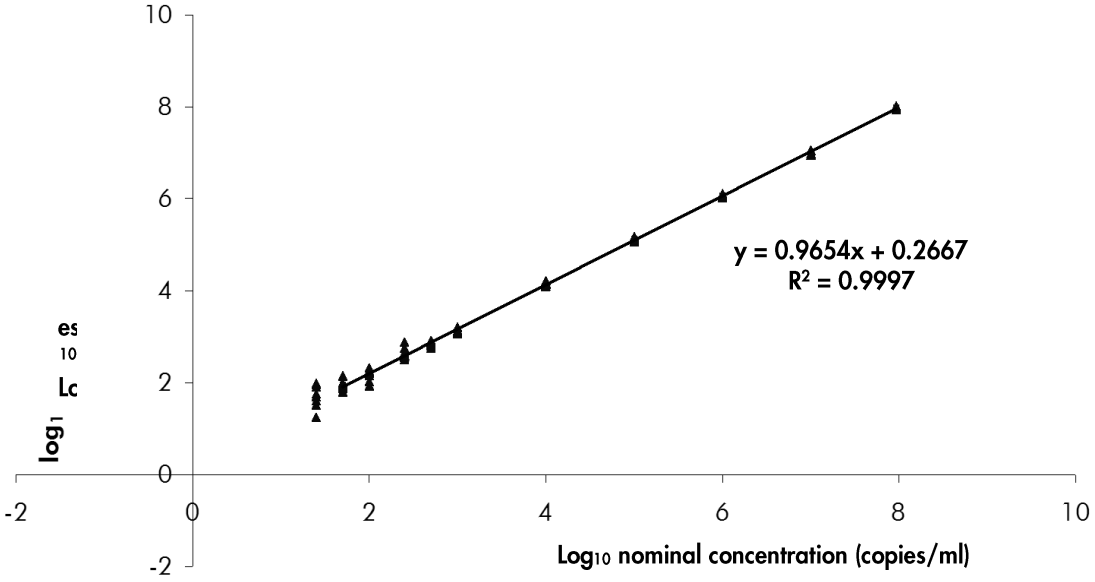


**Figure 2. Linear range of the *artus* BK Virus QS-RGQ Kit (plasma).** Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

# Robustness – plasma

The verification of the robustness allows the determination of the total failure rate of the *artus* BK Virus QS-RGQ Kit. To verify the robustness, 30 BK virus negative samples of plasma were spiked with 80 copies/ml of BK virus material (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Cellfree1000_DSP protocol (extraction volume: 1 ml, elution volume: 60 µl), these samples were analyzed with the *artus* BK Virus QS-RGQ Kit. In addition, the robustness of the internal control was assessed by purification and analysis of the 30 spiked plasma samples. Inhibitions were not observed. Thus, the robustness of the *artus* BK Virus QS-RGQ Kit is ≥99%.

# Interfering substances – plasma

Bilirubin, hemoglobin and triglycerides showed no interference with the *artus* BK Virus QS-RGQ Kit at concentrations shown in Table 3.

**Table 3. Interfering substances in EDTA plasma samples**

| BK virus concentration (copies/ml) | Interfering substance | | $C_{T(BKV)}$ | | | $C_{T(BKV)IS} - C_{T(BKV)\ Control}$ |
|---|---|---|---|---|---|---|
| | Item | Concentration | Average $C_T$ | SD | CV (%) | Absolute |
| 270 | Bilirubin | 30 mg/dl | 33.52 | 0.29 | 0.87 | 0.19 |
| | Hemoglobin | 2 g/dl | 33.63 | 0.33 | 0.97 | 0.07 |
| | Triglyceride | 1 g/dl | 33.56 | 0.14 | 0.42 | 0.15 |
| | Albumin | 6 g/dl | 34.15 | 0.26 | 0.77 | 0.45 |
| | Control | - | 33.71 | 0.20 | 0.60 | – |

BKV: BK virus; CV: coefficient of variation; IS: interfering substance; SD: standard deviation

# Clinical evaluation – plasma

The clinical performance of the *artus* BK Virus QS-RGQ Kit was evaluated by testing clinical specimens and analyzing the findings against the results from a comparable method. A total of 159 specimens of EDTA plasma collected from BK virus infected patients as well as from negative controls were tested with the *artus* BK Virus QS-RGQ Kit and the comparator method at an external site. The results were analyzed in two parts: part one was a categorical agreement analysis of positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA), see Table 4; part two was an analysis of the results from a total of 101 EDTA plasma samples that fell within the common assay dynamic range using Passing-Bablok and Deming regression analyses, see Figure 3.

**Table 4. Clinical performance study data for EDTA plasma samples**

| Measure of agreement | Frequencies | Percent agreement | Clopper-Pearson (exact) binomial lower two-sided 95% confidence limit | Clopper-Pearson (exact) binomial upper two-sided 95% confidence limit |
|---|---|---|---|---|
| Overall percent agreement | 159/159 | 100.00 | 97.71 | 100.00 |
| Positive percent agreement | 99/99 | 100.00 | 96.34 | 100.00 |
| Negative percent agreement | 60/60 | 100.00 | 94.04 | 100.00 |

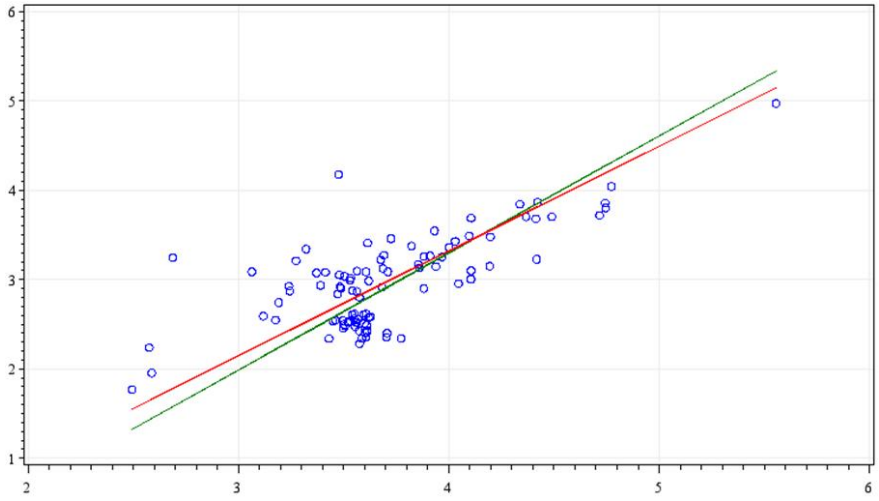


**Figure 3. Regression plot with Passing-Bablok and Deming lines (plasma).** Samples that were between the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ) for both kits were included in the analysis.

# Analytical sensitivity – urine 800 µl

For urine, the analytical sensitivity in consideration of the purification of the *artus* BK Virus QS-RGQ Kit was determined using a dilution series of BKV material from 316 to nominal 0.316 BKV copies/ml spiked in urine specimens. These were subjected to DNA extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Complex800_DSP protocol (extraction volume: 800 µl, elution volume: 60 µl). Each of the 10 dilutions was analyzed with the *artus* BKV QS-RGQ Kit on 4 different days in 4 runs with 11 replicates each. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Figure 4. The analytical detection limit in consideration of the purification of the *artus* BK Virus QS-RGQ Kit in combination with the Rotor-Gene Q is 78.5 copies/ml ($p = 0.05$). This means that there is a 95% probability that 78.5 copies/ml will be detected.

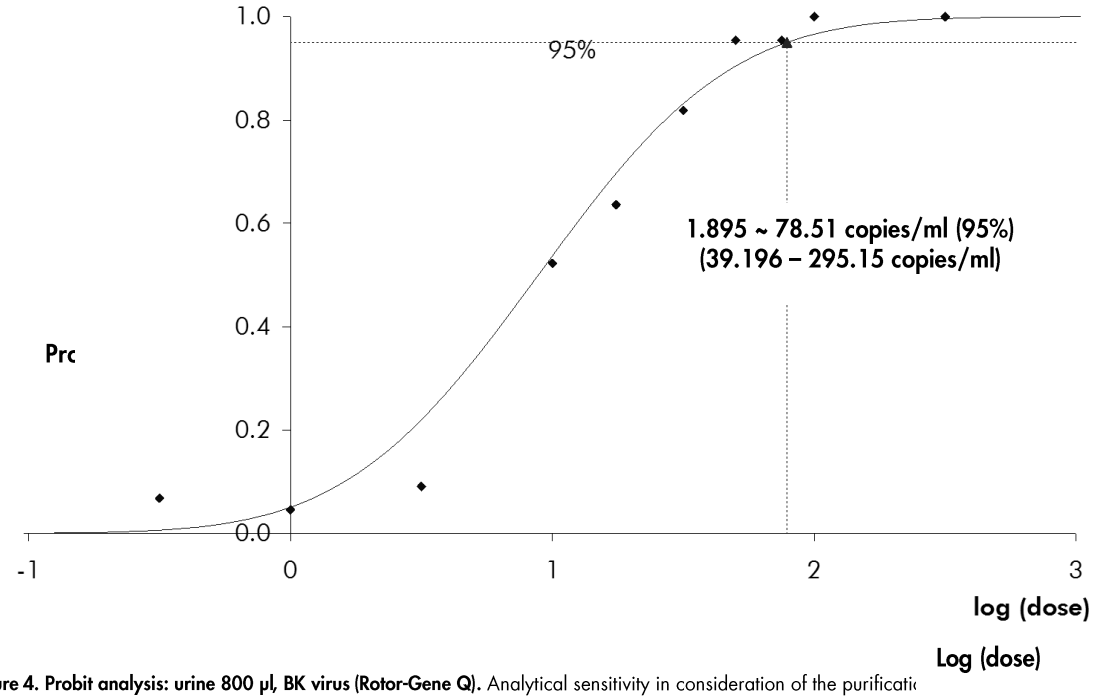


**Figure 4. Probit analysis: urine 800 µl, BK virus (Rotor-Gene Q).** Analytical sensitivity in consideration of the purificatic QIAsymphony DSP Virus / Pathogen Midi Kit) of the *artus* BK Virus QS-RGQ Kit on Rotor-Gene Q.

# Specificity – urine 800 µl

The specificity of the *artus* BK Virus QS-RGQ Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The detectability of all relevant genotypes has thus been ensured by a database alignment.

# Linear range – urine 800 µl

The linear range in consideration of the purification of the *artus* BK Virus QS-RGQ Kit was determined by analyzing a dilution series of BKV material ranging from 1.00 x $10^9$ copies/ml to 2.50 x $10^1$ copies/ml in urine. The purification was carried out in replicates (n = 4 for concentrations ≥1.00 x $10^8$ copies/ml; n = 8 for concentrations <1.00 x $10^8$ copies/ml) using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Complex800_DSP protocol (extraction volume: 800 µl, elution volume: 60 µl). Each of the samples was analyzed using the *artus* BK Virus QS-RGQ Kit. The linear range in consideration of the purification of the *artus* BK Virus QS-RGQ Kit has been determined to cover concentrations from 1.00 x $10^2$ copies/ml to 1.00 x $10^9$ copies/ml for urine (Figure 5).

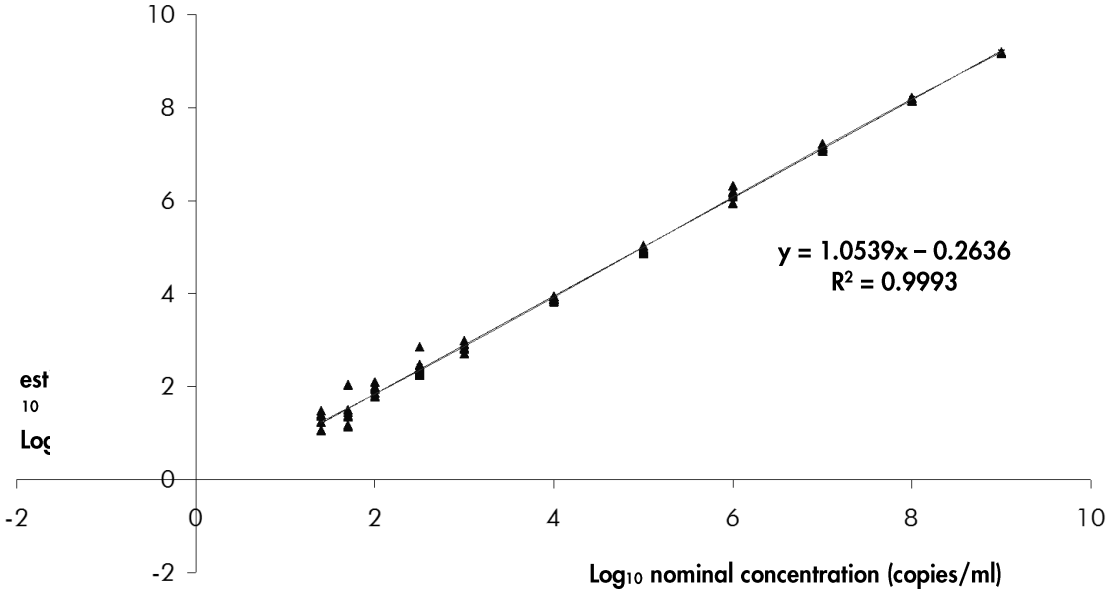


**Figure 5. Linear range of the *artus* BK Virus QS-RGQ Kit (urine 800 µl).** Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

# Robustness – urine 800 µl

The verification of the robustness allows the determination of the total failure rate of the *artus* BK Virus QS-RGQ Kit. To verify the robustness, 30 BK virus negative samples of urine were spiked with 236 copies/ml of BK virus material (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Complex800_DSP protocol (extraction volume: 800 µl, elution volume: 60 µl), these samples were analyzed with the *artus* BK Virus QS-RGQ Kit. In addition, the robustness of the internal control was assessed by purification and analysis of the 30 spiked urine samples. Inhibitions were not observed. Thus, the robustness of the *artus* BK Virus QS-RGQ Kit is ≥99%.

# Precision – urine 800 µl

Precision data in consideration of the purification of the *artus* BK Virus QS-RGQ Kit was collected using BKV material with a concentration of $1.125 \times 10^3$ copies/ml spiked in urine specimens. Testing was performed using the QIAsymphony DSP Virus/Pathogen Kit in combination with the Complex800_DSP protocol (extraction volume: 800 µl, elution volume: 60 µl). Testing was performed on 36 replicates using a matrix of various batches of the QIAsymphony DSP Virus/Pathogen Kit and the *artus* BK Virus QS-RGQ Kit. Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 0.97% ($C_T$) or 28.42% (concentration), and 2.61% ($C_T$) for the detection of the internal control (Tables 5 and 6). These values are based on the totality of all single values of the determined variabilities in consideration of the purification.

**Table 5. Precision data (total variance) on basis of the $C_T$ values**

| | Standard deviation | Variance | Coefficient of variation (%) |
|---|---|---|---|
| BK virus ($1.125 \times 10^3$ copies/ml) | 32.32 | 0.31 | 0.97 |
| Internal control (BK virus, $1.125 \times 10^3$ copies/ml) | 25.09 | 0.65 | 2.61 |

**Table 6. Precision data (total variance) on basis of the quantitative results (in copies/ml)**

| | Mean | Standard deviation | Coefficient of variation (%) |
|---|---|---|---|
| BK virus ($1.125 \times 10^3$ copies/ml) | $7.98 \times 10^2$ | $2.27 \times 10^2$ | 28.42 |

# Interfering substances – urine 800 µl

Interference testing was conducted on a selection of endogenous substances. Interference with the *artus* BK Virus QS-RGQ Kit was not seen for the substances listed in Table 7 at the concentrations given.

**Table 7. Interfering substances in EDTA plasma samples**

| BK virus concentration (copies/ml) | Interfering substance | | $C_{T(BKV)}$ | | | $\Delta C_{TIS - Control}$ |
|---|---|---|---|---|---|---|
| | Item | Concentration | Mean $C_T$ | SD | CV (%) | Absolute |
| 785 | Protein (HAS) | 1 mg/ml | 32.71 | 0.45 | 1.38 | –0.19 |
| | Glucose | 10 mg/ml | 32.56 | 0.12 | 0.37 | –0.34 |
| | gDNA | 35 ng/sample | 32.89 | 0.31 | 0.94 | –0.02 |
| | gDNA | 350 ng/sample | 32.86 | 0.22 | 0.67 | –0.05 |
| | Erythrocytes | 10 µg/sample | 32.16 | 1.36 | 4.22 | –0.75 |
| | Control | – | 32.91 | 0.57 | 1.72 | – |

BKV: BK virus; CV: coefficient of variation; gDNA: genomic DNA; IS: interfering substance; SD: standard deviation

# Clinical evaluation – urine 800 µl

The clinical performance of the *artus* BK Virus QS-RGQ Kit was evaluated by testing clinical specimens and analyzing the findings against the results from a comparable method. A total of 154 urine specimens collected from BK virus infected patients as well as from negative controls were tested with the *artus* BK Virus QS-RGQ Kit and the comparator method at an external site. The results were analyzed in two parts: part one was a categorical agreement analysis of PPA, NPA and OPA, see Table 8; part two was an analysis of the results from a total of 90 urine samples that fell within the common assay dynamic range using Passing-Bablok and Deming regression analyses, see Figure 6.

**Table 8. Clinical performance study data for urine samples**

| Measure of agreement | Frequencies | Percent agreement | Clopper-Pearson (exact) binomial lower two-sided 95% confidence limit | Clopper-Pearson (exact) binomial upper two-sided 95% confidence limit |
|---|---|---|---|---|
| Overall percent agreement | 150/154 | 97.40 | 93.48 | 99.29 |
| Positive percent agreement | 97/100 | 97.00 | 91.48 | 99.38 |
| Negative percent agreement | 53/54 | 98.15 | 90.11 | 99.95 |

**Note:** In Table 8, discrepancies in the results were only seen with samples that contained viral loads close to the limit of detection (LOD).

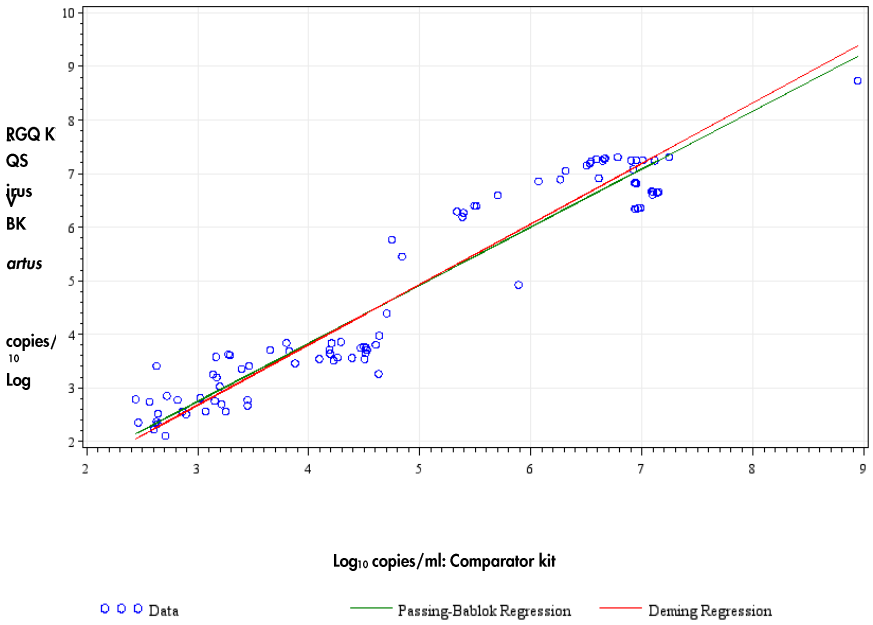


**Figure 6. Regression plot with Passing-Bablok and Deming lines (urine).** Samples that were between the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ) for both kits were included in the analysis.

# Analytical sensitivity – urine 400 µl

For urine, the analytical sensitivity in consideration of the purification of the *artus* BK Virus QS-RGQ Kit was determined using a dilution series of BKV material from 1000 to nominal 3.16 BKV copies/ml spiked in urine specimens. These were subjected to DNA extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Complex400_DSP protocol (extraction volume: 400 µl, elution volume: 60 µl). Each of the 8 dilutions was analyzed with the *artus* BKV QS-RGQ Kit on 4 different days in 4 runs with 11 replicates each. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Figure 7. The analytical detection limit in consideration of the purification of the *artus* BK Virus QS-RGQ Kit in combination with the Rotor-Gene Q is 81.83 copies/ml ($p = 0.05$). This means that there is a 95% probability that 81.83 copies/ml will be detected.

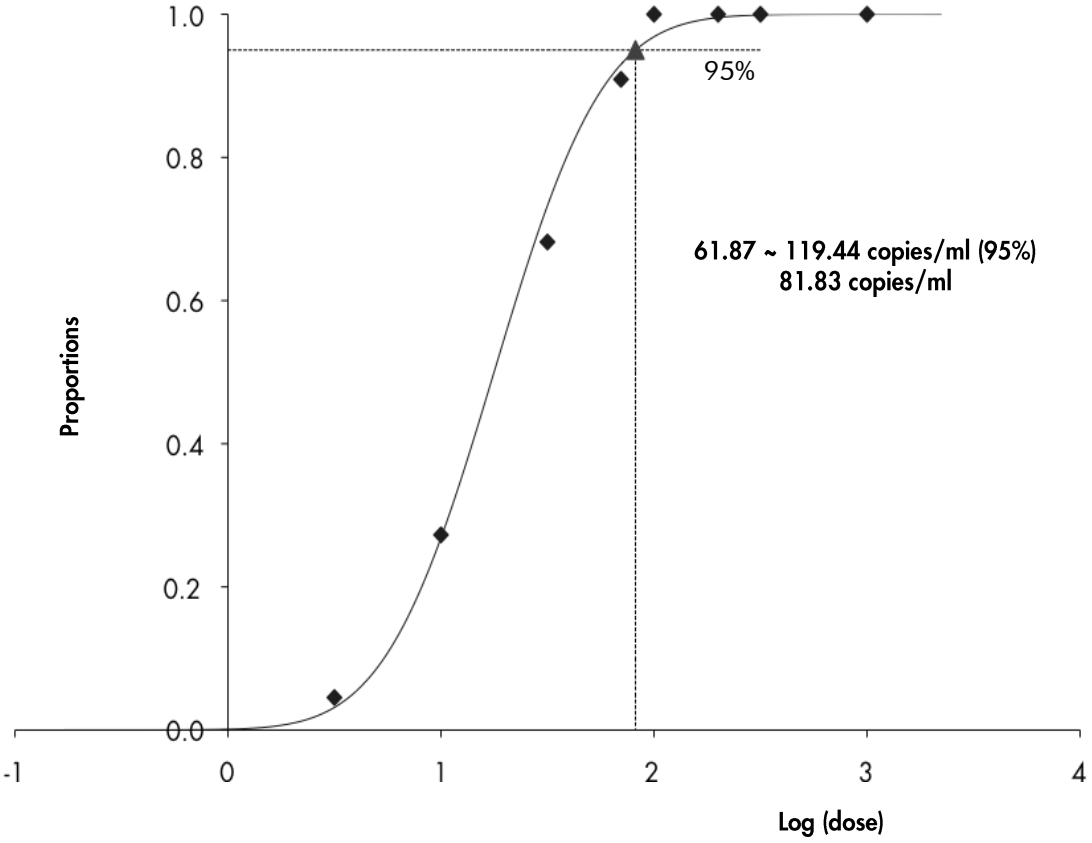


**Figure 7. Probit analysis: urine 400 µl, BK virus (Rotor-Gene Q).** Analytical sensitivity in consideration of the purification (urine, using the QIAsymphony DSP Virus/Pathogen Midi Kit) of the *artus* BK Virus QS-RGQ Kit onRotor-Gene Q.

# Linear range – urine 400 µl

The linear range in consideration of the purification of the *artus* BK Virus QS-RGQ Kit was determined by analyzing a dilution series of BKV material ranging from 1.00 x $10^9$ copies/ml to 2.50 x $10^1$ copies/ml in urine. The purification was carried out in replicates (n = 4 for concentrations ≥1.00 x $10^8$ copies/ml; n = 8 for concentrations <1.00 x $10^8$ copies/ml) using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Complex400_DSP protocol (extraction volume: 400 µl, elution volume: 60 µl). Each of the samples was analyzed using the *artus* BK Virus QS-RGQ Kit. The linear range in consideration of the purification of the *artus* BK Virus QS-RGQ Kit has been determined to cover concentrations from 2.5 x $10^2$ copies/ml to 1.00 x $10^9$ copies/ml for urine (Figure 8).

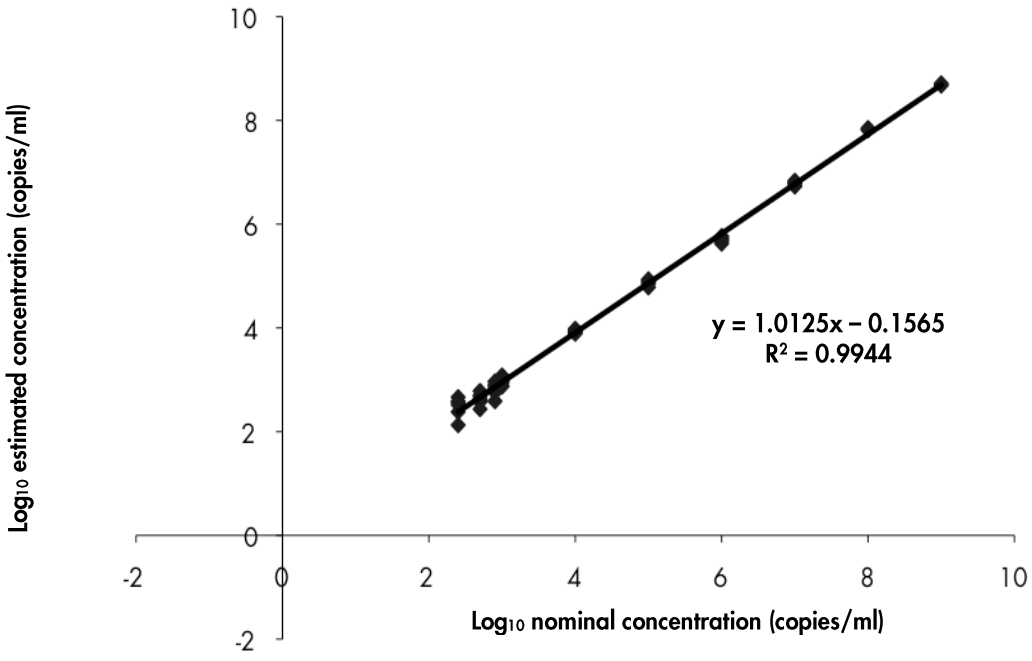


**Figure 8. Linear range of the *artus* BK Virus QS-RGQ Kit (urine 400 µl).** Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

# Robustness – urine 400 µl

The verification of the robustness allows the determination of the total failure rate of the *artus* BK Virus QS-RGQ Kit. To verify the robustness, 30 BK virus negative samples of urine were spiked with 245 copies/ml of BK virus material (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Complex400_DSP protocol (extraction volume: 400 µl, elution volume: 60 µl), these samples were analyzed with the *artus* BK Virus QS-RGQ Kit. In addition, the robustness of the internal control was assessed by purification and analysis of the 30 spiked urine samples. Inhibitions were not observed. Thus, the robustness of the *artus* BK Virus QS-RGQ Kit is ≥99%.

# Precision

The precision data of the *artus* BK Virus QS-RGQ Kit allow determination of the total variance of the assay. The total variance consists of the intra-assay variability (variability of multiple results of samples of the same concentration within one experiment), the inter-assay variability (variability of multiple results of the assay generated on different instruments of the same type by different operators within one laboratory) and the inter-batch variability (variability of multiple results of the assay using various batches). The data obtained were used to determine the standard deviation, the variance and the coefficient of variation for the pathogen specific and the internal control PCR.

Analytical precision data of the *artus* BK Virus QS-RGQ Kit (without consideration of the purification) were collected using the quantitation standard (QS) of the lowest concentration (QS 4; 10 copies/µl). Testing was performed with 8 replicates. The precision data were calculated on basis of the $C_T$ values of the amplification curves ($C_T$: threshold cycle, see Table 9). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 2.11% ($C_T$), and 3.59% ($C_T$) for the detection of the internal control. These values are based on the totality of all single values of the determined variabilities.

**Table 9. Precision data on basis of the $C_T$ values**

| | $C_T$ value | Standard deviation | Coefficient of variation (%) |
|---|---|---|---|
| Intra-assay variability: BK Virus RGQ QS 4 | 29.45 | 0.17 | 0.56 |
| Intra-assay variability: Internal control | 24.31 | 0.12 | 0.49 |
| Inter-assay variability: BK Virus RGQ QS 4 | 29.42 | 0.25 | 0.85 |
| Inter-assay variability: Internal control | 23.30 | 0.77 | 3.30 |
| Inter-batch variability: BK Virus RGQ QS 4 | 30.31 | 0.64 | 2.10 |
| Inter-batch variability: Internal control | 22.53 | 0.40 | 1.78 |
| Total variance: BK Virus RGQ QS 4 | 29.80 | 0.63 | 2.11 |
| Total variance: Internal control | 23.12 | 0.83 | 3.59 |

## Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* BK Virus QS-RGQ Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

## Cross-contamination

Absence of cross-contamination between samples for the entire workflow was proven by the correct detection of all known positive and negative samples in alternating positions (checkerboard pattern) for a representative *artus* QS-RGQ system.

Related products and ordering information are listed in the handbook for the *artus* BK Virus QS-RGQ Kit

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN® kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.