

Impact of two different commercial DNA extraction methods on BK virus viral load

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Summary

Background and aim: BK virus, a member of human polyomavirus family, is a worldwide distributed virus characterized by a seroprevalence rate of 70-90% in adult population. Monitoring of viral replication is made by evaluation of BK DNA by quantitative polymerase chain reaction. Many different methods can be applied for extraction of nucleic acid from several specimens. The aim of this study was to assess the impact of two different DNA extraction procedure on BK viral load.

Materials and methods: DNA extraction procedure including the Nuclisens easyMAG platform (bioMerieux, Marcy l'Etoile, France) and manual QIAGEN extraction (QIAGEN Hilden, Germany). BK DNA quantification was performed by Real Time TaqMan PCR using a commercial kit.

Result and discussion: The samples capacity, cost and time spent were compared for both systems. In conclusion our results demonstrate that automated nucleic acid extraction method using Nuclisense easyMAG was superior to manual protocol (QIAGEN Blood Mini kit), for the extraction of BK virus from serum and urine specimens.

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Introduction

BK virus, a member of human polyomavirus family, is a worldwide distributed virus characterized by a seroprevalence rate of 70-90% in adult population (6,12).

BK virus establishes latency in uroepithelial cell, in B cell, brain, spleen and probably in other tissues. The virus can become reactivated in setting of immunodeficiency and result in cellular damage and organ dysfunction (3,7,9,11).

Monitoring of viral replication is made by evaluation of BK DNA by quantitative polymerase chain reaction (PCR). The success and reliability of nucleic acid sequence amplification require efficient unbiased procedure of extraction (2,15). A high-quality nucleic acid extract is expected to be free of amplification inhibitors and other substance that might affect enzyme substrates (10).

Many different methods can be applied for extraction of nucleic acid from several specimens. Common extraction procedures include phenol chloroform purification or the use of commercially available kits (5).

The aim of this study was to assess the impact of two different DNA extraction procedures including the Nuclisens easyMAG platform (bioMerieux, Marcy l'Etoile, France) and manual QIAGEN extraction, on viral load detection in serum and urine samples.

Materials and Methods

Subjects

A total of 52 specimens of which 28 urine and 24 serum samples were recruited by transplant patient afferent to Ospedale Maggiore

della Carità (Novara, Northern Italy), twenty-four men and nine female with median age 57.18 ± 12.8 years. Ten sera and urine was obtained from healthy control subjects with median age 55.3 ± 12.4 referred to AVIS blood donor centre S. Anna hospital. Each healthy control completed a questionnaire to verify a good health status.

Manual method (QIAGEN DNA blood MiniKit)

The extraction procedure was performed with QIAGEN DNA blood MiniKit according to the manufacturer's instruction.

A maximum of 10 samples can be manually processed in each extraction.

Briefly: 20 μ L of protease and then 200 μ L of AL buffer were added to 200 μ L of each sample and extraction tubes were vortexed and incubated for 10' at 56°C. Two hundred μ L of 96% ethanol was added and, the mixture was transferred to a QIAamp column and centrifuged for 1' at 6000 g.

The column was put in a new collection tube, 500 μ L AW1 buffer was added and centrifuged for 1' at 6000 g. This procedure was repeated with 500 μ L AW2 buffer. To remove all ethanol from the column it was put in a new collection tube and then subjected to a dry spin for 1' at full speed. Elution was performed by adding 50 μ L EL buffer for serum samples and 200 μ L for urine samples, incubating for 5' at room temperature following by centrifugation for 1' at 6000 g.

Automated method (easyMAG nuclisens extraction)

Extraction with easyMAG was done according to the manufacturer's recommendations. A maximum of 24 samples can be processed in each extraction. A total of 1000 μ L of urine and 500 μ L of serum samples were placed in the disposable sample vessel and then were loaded onto the extractor. After the initial lysis incubation, 100 μ L of magnetic silica, prepared as recommended by the manufacturer, was added to each sample, and the extractor was restarted.

Samples were eluted in 25 μ L.

Real-time PCR assays

The quality of extracted DNA was tested in PCR amplification. BK DNA quantification was performed by Real Time TaqMan. PCR using a commercial kit (Elitechgroup, Milano Italy) with the 7300 Real Time PCR System (Applied Biosystems Monza, Italy). According to the manufacturer's instruction PCR amplification were set up in reaction volume of 25 μ L that contained 5 μ L of extracted sample, or negative control (sterile double-distilled H₂O) or standard plasmid dilutions (100;1000;10000;100000 copies/ μ L).

Clinical specimens were processed with the following thermic profile: 50°C for 2 min (decontamination) and one cycle of initial denaturation at 95°C for 10 min followed by 45 cycles of 15s at 95°C (denaturation), and 1 min at 60°C (annealing and extension).

Each sample was subjected to simultaneous TaqMan PCR for house-keeping gene human β -globin. Results were considered acceptable only in the presence of β -globin positivity. The results were classified as negative, high positive (>100,000 copies/mL) and low positive (<100,000 copies/mL) or invalid if the internal control was not detected.

Results and Discussion

The aim of this study was to compare two different DNA extraction methods in order to establish their relative effectiveness for extracting viral DNA from serum and urine samples.

A total of 72 clinical specimens, which 38 urine and 34 serum samples, were used to comparative analysis of two different extraction procedures including manual QIAGEN extraction method and automated

Nuclisens easyMAG platform (bioMerieux, Marcy l'Etoile, France). According to the manufacturer's instruction, while manual extraction DNA kit used a silica gel membranes and enzymatic digestion with protease, the automated extraction procedure is based on Boom method. This is a mechanism by which DNA selectively binds onto glass particles (silica) in the presence of high concentration of chaotropic agent, such as guanidinium thiocyanate, while contaminant such as proteins, carbohydrates and ions do not, and subsequent washing and elution of nucleic acid (2).

After the extraction procedures were completed, the DNA was immediately quantified by Real Time Taq PCR using a commercial kit (Elitechgroup, Milano Italy).

Each samples was subjected to simultaneous TaqMan PCR for house-keeping gene human β -globin.

Results were considered acceptable only in presence of β -globin positivity.

The use of internal control could exclude false-negative results due to inhibitors present in clinical samples. For examples in blood samples the prosthetic group of haemoglobin released from erythrocytes following haemolysis, reversibly bind to Taq polymerase inhibiting its activity. Furthermore, there are different materials inhibit PCR by direct effect on Taq polymerase as follow: heparin, phenol, denatured albumin etc. (2). Urine has been found to be difficult specimens for PCR-based amplification, due to the presence of many possible inhibitors (4,16).

The results were classified as negative, high positive and low positive or invalid if the internal control was not detected. No sample was invalid.

Overall, of 34 serum samples extracted with two different procedures, results were concordant for 30 samples and discordant for 4, with a positive and negative results found by different methods for same samples. In particular, the results were concordant for positive samples with high titre of BK viral load and for negative samples. Considering discordant results, the Nanogen kit detected 20 samples extracted with automated method as low positive, in confront off only 16 samples extracted with manual method. The remainder 4 samples extracted with manual procedure were detected as negative.

Furthermore, considering 38 urine samples, the results were concordant for 28 samples extracted with both methods: 10 samples presents a high BK viral load, 10 specimens were negative and finally, 8 urine samples contain low BK viral load. The results were discordant for 10 urine samples. In particular, PCR Real Time was negative for 20 specimens extracted with Qiagen blood kit including 10 urine samples with low viral load, when the urine samples were extracted with Nuclisens easyMAG method (Table 1). The results were discordant for 4 serum samples. In particular, this samples resulted positive with low viral load when extracted with Nuclisens easyMAG method and negative when extracted with Qiagen blood kit (Table 2).

The differences in viral load between urine and serum samples extracted with manual and automated procedures respectively, could be explained by a operational complexity, defined as a number of manipulation required to obtain an extracted samples that was highest for QIAamp DNA Blood Mini kit (5). Most authors cite the

Table 1. Discordant results between urine samples extracted with automated method and manual procedure, tested in PCR amplification. BK DNA quantification was performed by Real Time TaqMan. PCR using a commercial kit (Elitechgroup, Milano Italy).

	High positive	Low positive	Negative	Total
Nuclisens easy MAG	10	18	10	38
QIAGEN Blood Mini kit	10	8	20	38

Table 2. Discordant results between urine samples (or serum samples) extracted with automated method and manual procedure, tested in PCR amplification. BK DNA quantification was performed by Real Time TaqMan. PCR using a commercial kit (Elitechgroup, Milano Italy).

	High positive	Low positive	Negative	Total
Nuclisens easy MAG	4	20	10	34
QIAGEN Blood Mini kit	4	16	14	34

Table 3. Comparison of cost, processing time, and additional materials required for the two extraction procedures.

	Nuclisens easy MAG	QIAGEN Blood Mini kit
Cost per extraction in Euro	11	4.38
Approximate processing time	50 min	90 min
Consumables/additional reagents	None	Tubes, ethanol

easyMAG as a user-friendly instrument, requiring little manipulation (5,14). The number of manual specimens transfers was different in each methods. The manual procedure required three transfers, while the automated methods required two transfers, this results in a greater chance of error as nucleic acid loss, and the results would be a false negative. Furthermore, operational complexity in QIAamp DNA Blood Mini kit, may be cause false positive results due to cross contamination of negative specimens by strongly positive specimens.

These risks can contribute to incorrect decisions with potentially severe consequences for the patients. The evolution of BKV replication represents the basic strategy to early predict the onset of BKVAN, and to asses the clinical course thereof and monitor the response to treatment, too (1,7,8,13).

The samples capacity, cost and time spent were compared for both systems (Table 3). The Nuclisens easyMAG, was able to process significantly more samples per run than QIAamp method. One to 24 samples can be analyzed in one run and DNA and RNA extraction can be performed within the same run, while a maximum of 10 samples can be manually processed in each extraction. The automated extraction method can be applied to a broad range of different specimens as blood, serum, urine etc.

The turnaround time for nucleic acid extraction with Nuclisens easyMAG was 50 min for 24 samples including 10 min of incubation with lysis buffer and about 10 min of hands-on time. Nucleic acid extraction of 10 samples with QIAGEN takes 90 min starting from addition of the enzyme.

The cost/sample of automated extraction method is higher than manual extraction procedure, as shown in Table 3.

Conclusions

In conclusion our results demonstrate that automated nucleic acid extraction method using Nuclisens easyMAG was superior to manual protocol (QIAGEN Blood Mini kit), for the extraction of BKV from serum and urine specimens, even though Nuclisens easyMAG was more expensive than manual extractor protocol.

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