

# Evaluation of six methods for extraction and purification of viral DNA from urine and serum samples

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## SUMMARY

The sensitivity and reliability of PCR for diagnostic and research purposes require efficient unbiased procedures of extraction and purification of nucleic acids. One of the major limitations of PCR-based tests is the inhibition of the amplification process by substances present in clinical samples. This study used specimens spiked with a known amount of plasmid pBKV (ATCC 33-1) to compare six methods for extraction and purification of viral DNA from urine and serum samples based on recovery efficiency in terms of yield of DNA and percentage of plasmid pBKV recovered, purity of extracted DNA, and percentage of inhibition. The most effective extraction methods were the phenol/chloroform technique and the silica gel extraction procedure for urine and serum samples, respectively. Considering DNA purity, the silica gel extraction procedure and the phenol/chloroform method produced the most satisfactory results in urine and serum samples, respectively. The presence of inhibitors was overcome by all DNA extraction techniques in urine samples, as evidenced by semiquantitative PCR amplification. In serum samples, the lysis method and the proteinase K procedure did not completely overcome the presence of inhibitors.

KEY WORDS: PCR, DNA extraction, BKV, PCR inhibitors

Received February 8, 2006

Accepted March 14, 2006

## INTRODUCTION

Molecular biology techniques represent a powerful tool for nucleic acid analysis, enabling the detection of even a single genomic copy and are widely used for diagnostic and research purposes. The target nucleic acid sequences may be amplified by PCR and the product (amplicon) detected in ethidium bromide-stained gel or by colorimetric enzyme immunoassay. In particular, the ability of PCR to amplify nucleic acid

sequences has allowed the determination of presence and quantification of specific viral genes and sequences in biological matrices. Indeed, the detection of nucleic acid sequences from many viruses, including polyomavirus BK, in serum and urine samples for routine diagnostics is currently performed by PCR in most laboratories. The success and reliability of nucleic acid sequences amplification require efficient unbiased procedures of extraction and purification from other microorganism components, biological substances, and contaminating materials which could function as PCR inhibitors (Wiedbrauk *et al.*, 1995). An efficient unbiased method of DNA extraction that yields pure, high-quality DNA is crucial for the success of PCR and sequencing reactions and the subsequent treatment of disease (McOrist *et al.*, 2002). One of the major limitations of PCR-based tests is the inhi-

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bition of the nucleic acid amplification process by substances present in clinical samples. For example, DNase or RNase can degrade target nucleic acid and/or oligonucleotide primers (Mullen *et al.*, 1990); EDTA can chelate divalent cations such as  $Mg^{2+}$  that are essential for the activity of the thermostable enzyme *Taq* polymerase (Greenfield *et al.*, 1993); different materials such as heparin (Holodniy *et al.*, 1991; Izraeli *et al.*, 1991), phenol (Katcher *et al.*, 1994), denatured albumin (Vandenvelde *et al.*, 1993), polyamines (Ahokas *et al.*, 1993), polysaccharides (Shioda *et al.*, 1987), and calcium alginate (Wadowsky *et al.*, 1994) inhibit PCR by direct effect on *Taq* polymerase. Haem, the prosthetic group of hemoglobin released from erythrocytes following hemolysis and frequently associated with blood sampling, reversibly binds to *Taq* polymerase inhibiting its activity (Byrnes *et al.*, 1975; Levere *et al.*, 1991; Mercier *et al.*, 1990; Tsutsui and Mueller, 1987). As little as 1% (vol/vol) blood completely inhibits *Taq* polymerase activity (Panaccio and Lew, 1991). Urine has been found to be a particularly difficult specimen for PCR-based amplification, due to the presence of many possible inhibitors (Demmler *et al.*, 1988; Yamaguchi *et al.* 1992). For example, concentrations of 20 mM urea and above are inhibitory for PCR (Khan *et al.*, 1991). Several studies have been devoted to the development of sample pretreatments to obtain adequate specimens (Lantz *et al.*, 2000) or to enhance the efficiency of PCR using an alternative thermostable DNA polymerase more resistant to inhibitors (Abu Al-Soud and Rådström, 1998; Katcher and Schwartz, 1994; Panaccio and Lew, 1991; Wiedbrauk *et al.*, 1995) or amplification facilitators such as bovine serum albumin, the single-stranded DNA binding T4 gene 32 protein (gp32), organic solvents, and proteinase inhibitors (Abu Al-Soud and Rådström, 2000; Akane *et al.*, 1994; Chandler *et al.*, 1998; de Lomas *et al.*, 1992; Demeke and Adams, 1992; Gilgen *et al.*, 1995; Izraeli *et al.*, 1991; Kreader, 1996; Pomp and Medrano, 1991; Powell *et al.*, 1994; Vandenvelde *et al.*, 1993). Many lysis and DNA extraction methods have been described in literature (Behzadbehbahani *et al.*, 1997; Bowtell, 1987; Ciulla *et al.*, 1988). The phenol-chloroform extraction procedure for DNA (Sambrook *et al.*, 1989) followed by ethanol precipitation has been

traditionally used to obtain nucleic acids from cells and microorganisms. In addition to separation and purification from contaminating materials, this method minimizes the inhibition of nucleic acid amplification by PCR in clinical samples (Khan *et al.*, 1991; Ruano *et al.*, 1992; Wiedbrauk *et al.*, 1995). The aim of this study was to compare six different DNA extraction procedures from urine and serum samples for subsequent PCR analysis. Using specimens spiked with known amounts of BK virus DNA, the following extraction protocols were compared: a commercial Kit (Extragen), a lysis extraction procedure which uses a lysis buffer, a lysis extraction procedure which employs a lysis buffer in conjunction with a hot pretreatment, a phenol/chloroform extraction followed by ethanol precipitation, a lysis extraction which utilizes a guanidium thiocyanate/silica matrix method, and a lysis extraction procedure which utilizes a lysis buffer in conjunction with proteinase K. The six extraction protocols were evaluated on the basis of the following criteria: total yield of DNA, purity of the DNA recovered, and presence of PCR-inhibitory substances. In addition, physical, chemical, and enzymatic elements were briefly analyzed.

## MATERIALS AND METHODS

### Experimental design

In order to evaluate DNA extraction and purification procedures, a known amount of BK virus DNA was added to biological samples and the amount recovered after extraction and purification was determined. Serum and urine specimens were obtained from 10 healthy volunteers and stored at  $-30^{\circ}C$ . All specimens were previously tested for BK virus DNA by nested PCR and resulted negative.

BK virus DNA-negative sera and urines were spiked with a known amount (400 ng) of plasmid pBKV 33-1 (obtained from the American Type Culture Collection [Rockville, USA]), containing the whole sequence of the polyomavirus BK. The spiked samples were then extracted by the six different extraction methods and tested by semiquantitative PCR. Extraction procedures were performed for each specimen in quintuplicate, giving a total number of 50 extractions

from urine samples and 50 extractions from serum samples. Evaluation of extraction protocols was based on three features:

- 1) recovery efficiency in terms of yield of DNA and percentage of plasmid pBKV recovered in each sample, as determined using a spectrophotometer;
- 2) purity of extracted DNA based on spectrophotometric readings of the 260/280 nm-absorbance ratio (pure DNA has a 260/280 nm-absorbance ratio equal to 1.8);
- 3) presence of inhibitors, as estimated by a PCR inhibition assay in which 1 ng of extracted DNA were used as template for semiquantitative PCR amplification of BK virus Large T antigen.

One target-free control for every sample was included in each test run. In order to reduce the likelihood of contamination, specimen extraction and analysis of amplified products were performed in separate rooms (Espy *et al.*, 1995). In addition, positive-displacement pipettes, together with disposable gowns and gloves were used as previously recommended (Kwok and Higuchi, 1989).

### DNA extraction methods

#### *Protocol A: Extragen Kit*

(Amplimedical s.p.a., Turin, Italy)

The extraction procedure was performed according to the manufacturer's instructions. Briefly, 250  $\mu$ l of each sample were added to 250  $\mu$ l of lysis buffer in an extraction tube, vortexed, incubated for 10 min at 80°C, then centrifuged for 15 min at 13,000 rpm at room temperature. Seven hundred  $\mu$ l of the supernatant were mixed with 700  $\mu$ l of isopropanol, and centrifuged for 10 min at 13,000 rpm at room temperature. The supernatant was removed, and the pellet resuspended in 1 ml of 70% ethanol, vortexed, and centrifuged for 1 min at 13,000 rpm at room temperature. The supernatant was removed again and the remaining ethanol eliminated by a quick centrifuge spin. The pellet was air-dried for at least 30 min in a half-open tube, resuspended in 20  $\mu$ l of double-distilled H<sub>2</sub>O, and stored at -20°C prior to use.

#### *Protocol B: home-made extraction procedure #1*

Prior to DNA extraction, urine and serum samples were diluted two-fold in PBS 1X, boiled for

10 min, and then centrifuged for 15 min at 13,000 rpm at room temperature. A total of 250  $\mu$ l of supernatant of boiled samples was added to 250  $\mu$ l of lysis buffer (400 mM Tris-HCl pH 7.5, 500 mM NaCl, 50 mM EDTA, 1% SDS), incubated for 30 min, then centrifuged for 5 min at 13,000 rpm at room temperature. Four hundred  $\mu$ l of the supernatant were mixed with 400  $\mu$ l of isopropanol and incubated at -30°C for 15 min. After centrifugation for 5 min at 13,000 rpm at room temperature, the pellet was washed with 70% ethanol, centrifuged for 5 min at 13,000 rpm at room temperature, air-dried for at least 30 min, resuspended in 20  $\mu$ l of double-distilled H<sub>2</sub>O, and stored at -20°C prior to use.

#### *Protocol C: home-made extraction procedure #2.*

A total of 250  $\mu$ l of each sample was added to 250  $\mu$ l of lysis buffer (400 mM Tris-HCl pH 7.5, 500 mM NaCl, 50 mM EDTA, 1% SDS), incubated for 60 min at room temperature, then centrifuged for 10 min at 13,000 rpm at room temperature. Four hundred  $\mu$ l of the supernatant were mixed with 400  $\mu$ l of isopropanol and incubated at -30°C for 15 min. After centrifugation for 5 min at 13,000 rpm, the pellet was washed with 70% ethanol, centrifuged for 5 min at 13,000 rpm at room temperature, air-dried for at least 30 min, resuspended in 20  $\mu$ l of double-distilled H<sub>2</sub>O, and stored at -20°C prior to use.

#### *Protocol D: phenol/chloroform extraction procedure*

This home-made procedure was based on the classical phenol/chloroform extraction method. A total of 250  $\mu$ l of each sample was added to 250  $\mu$ l of buffer-saturated phenol/chloroform/isoamylalcohol (pH 7.5-7.8) and 25  $\mu$ l of 3M sodium acetate pH 5, vortexed for 1 min, incubated in ice for 15 min, and centrifuged for 10 min at 12,000 rpm at room temperature. The aqueous layer was transferred to a 1.5 ml screw-cap plastic tube containing 250  $\mu$ l of isopropanol and incubated at -20°C for 5 min. The sample was centrifuged (5 min, 12,000 rpm at 4°C) in order to precipitate DNA. The supernatant was removed, and the pellet resuspended in 500  $\mu$ l of 70% ethanol, vortexed and centrifuged for 2 min at 13,000 rpm at room temperature. The supernatant was removed again and the remaining ethanol eliminated by a quick centrifuge spin.

The pellet was air-dried in a half-open tube for at least 30 min, suspended in 20  $\mu$ l of TE (10 mM TrisCl, 1 mM EDTA pH 8) by vortexing, and stored at -20°C prior to use.

*Protocol E: Boom method (silica gel extraction procedure)*

A total of 150  $\mu$ l of each urine and serum sample was added to 1,350  $\mu$ l of lysis buffer (4.7 M guanidium thiocyanate, 46 mM Tris buffer pH 6.4, 20 mM EDTA, 1.2% w/v Triton X-100). Subsequently, nucleic acid extraction was performed by activated silica absorption, as described by Boom *et al.* (Boom *et al.*, 1990). Nucleic acid was eluted in 12  $\mu$ l of 1 mM Tris buffer (pH 8.5) and stored at -20°C prior to use.

*Protocol F: proteinase K extraction procedure*

A total of 250  $\mu$ l of each urine and serum sample was added to 500  $\mu$ l of lysis buffer (10 mM Tris-HCl pH 7.5, 5 mM MgCl<sub>2</sub>, 320 mM sucrose, 1% Triton x-100), and centrifuged at 13,000 rpm for 1 min. The pellet was suspended in 20  $\mu$ l of PK buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl<sub>2</sub>, 0.45% NP40, 0.45% Tween 20, Proteinase K 1.2  $\mu$ g) by vortexing, and incubated at 55 °C for 1 h. Subsequently, the Proteinase K was inactivated at 97 °C for 10 min. Nucleic acid was stored at -20°C prior to use.

### Semiquantitative PCR

The same PCR amplification was performed on 1 ng of each sample in order to detect pBKV DNA (ATCC 33-1), regardless of extraction method. Amplification steps, assay conditions, signal, and visualization steps were identical.

A fragment of the large T antigen gene of the BKV was amplified from plasmid pBKV with a semi-quantitative PCR. Briefly, a variable volume of extracted DNA (corresponding to 1 ng) and an external standard (four dilution containing 0.01, 0.1, 1 and 10 ng of pBKV) were added to reach 50  $\mu$ l of PCR solution containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin, 1 U *Taq* DNA polymerase (Sigma Chemical Co., St. Louis, Mo.), 200  $\mu$ M of each dNTP and 2.5 pmol of each primer. After an initial denaturation step of 5 min at 94 °C, the PCR amplification was carried out: 95 °C for 15 s, 48 °C for 30 s, 72 °C for 30 s for 28 cycle, then one cycle at 72 °C for 5 min. The sequences of the

oligonucleotide primers used were 5'-CTGGGT-TAAAGTCATGCT-3' nucleotide positions (nts) 2185-2202 and 5'-GGTAGAAGACCCTAAAGACT-3' nts 2589-2570 yielding a 385 bp product. (Ferrante *et al.*, 1995).

Amplification was carried out with a 9800 thermal cycler (Applied Biosystem, Monza, Italy). The amplified PCR products were detected by direct gel analysis. A 20  $\mu$ l sample of each amplification product was electrophoresed on a 2% agarose gel. The amplification products were quantified using a digital imaging system (Quantity One 1-D Analysis Software; BIO-RAD, USA). Samples were analyzed by comparing the imaged PCR results with the standard curve. The relative intensity values for each pBKV were related to copy number of pBKV for each dilution by linear regression.

## RESULTS

The aim of this study was to compare six different DNA extraction methods in order to ascertain their relative effectiveness for extracting viral DNA from serum and urine samples. Protocol A is a commercial extraction method (Extragen) based on a salting out procedure. Protocol B (home-made extraction procedure #1) is based on the use of a lysis buffer and on the coagulation of proteins present in the sample by boiling. Protocol C (home-made extraction procedure #2) simply utilizes a lysis buffer. Protocol D is based on classical standard phenol/chloroform extraction followed by ethanol precipitation. The Boom method employed in protocol E is based on the mechanism by which DNA selectively binds onto glass particles (silica) in the presence of high concentrations of a chaotropic agent, such as guanidium thiocyanate, while contaminant such as proteins, carbohydrates, and ions do not, and the subsequent washing and elution of nucleic acids. Protocol F is an extraction procedure in which lysis is obtained by enzymatic digestion with proteinase K.

Yield of DNA, percentage of plasmid pBKV recovered by employing the six protocols in all samples, purity of DNA extracted, based on readings of the 260/280 nm-absorbance ratio, and inhibition percentage are summarized in Tables 1 and 2. The DNA extracted by the six different

TABLE 1 - Comparison of six DNA extraction protocols used for urine samples.

Extraction protocol	Input	Output	% Recovery	Mean R 260/280	% Inhibition (semiquantitative results/ng amplified)
Protocol A	400	324 ± 15	81 ± 3.76	1.54	0 (1/1)
Protocol B	400	292 ± 64	73 ± 16	1.45	0 (1/1)
Protocol C	400	330 ± 37.4	82.5 ± 9.35	1.57	0 (1/1)
Protocol D	400	350 ± 46.9	87.5 ± 11.7	1.57	0 (1/1)
Protocol E	400	344 ± 19.7	86 ± 4.9	1.58	0 (1/1)
Protocol F	400	348 ± 40	87 ± 10	1.19	0 (1/1)

The pBKV DNA input (ng) is displayed in the first column. For each method, yield of DNA (mean output ± SD, ng), recovery percentage (mean ± SD), purity of DNA extracted based on spectrophotometric readings of the 260/280 nm-absorbance ratio (mean R 260/280), and inhibition percentage as evidenced by semiquantitative PCR amplification are displayed.

TABLE 2 - Comparison of six DNA extraction protocols used for serum samples.

Extraction protocol	Input	Output	% Recovery	Mean R 260/280	% Inhibition (semiquantitative results/ng amplified)
Protocol A	400	306 ± 93.5	76.5 ± 22.5	1.67	0 (1/1)
Protocol B	400	290 ± 95.2	72.5 ± 23.8	1.63	0 (1/1)
Protocol C	400	268 ± 76.9	67 ± 17.5	0.69	18 (0.8/1)
Protocol D	400	302 ± 60	75.5 ± 14.9	1.73	0 (1/1)
Protocol E	400	307 ± 67.7	76.6 ± 16.9	1.53	0 (1/1)
Protocol F	400	306 ± 58.5	76.5 ± 14.6	0.95	14 (0,86/1)

The pBKV DNA input (ng) is displayed in the first column. For each method, yield of DNA (mean output ± SD, ng), recovery percentage (mean ± SD), purity of DNA extracted based on spectrophotometric readings of the 260/280 nm-absorbance ratio (mean R 260/280), and inhibition percentage as evidenced by semiquantitative PCR amplification are displayed.

protocols was amplified by a semiquantitative PCR to determine the presence of inhibitors of the *Taq* polymerase (Figure 1). For urine samples (Table 1), all six methods successfully extracted BK virus DNA, as demonstrated by semiquantitative PCR amplification. Protocol D (phenol/chloroform extraction procedure) was the most effective extraction method with a mean yield of DNA of 350 ng recovered vs 400 ng of plasmid pBKV initially added, corresponding to a recovery percentage of 87.5%. Protocol B (home-made extraction procedure #1) exhibited the lowest mean yield of DNA recovered (i.e. 292 ng of plasmid pBKV vs 400 ng ini-

tially added), with a recovery percentage of 73%. DNA recovery percentages by protocols A, C, E and F were equal to 81%, 82.5%, 86% and 87%, respectively. For serum samples (Table 2), protocol E (silica gel extraction procedure) was the most effective method with a mean amount of DNA of 307 ng recovered vs 400 ng of plasmid pBKV initially added, corresponding to a recovery percentage of 76.6%. Protocol C (home-made extraction procedure #2) exhibited the lowest mean yield of DNA recovered (i.e. 268 ng of plasmid pBKV vs 400 ng initially added), corresponding to a recovery percentage of 67%. Protocols A, B, D and F exhibited intermediate

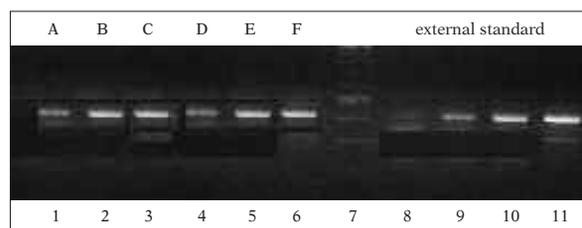


FIGURE 1 - Detection of pBKV DNA by semiquantitative PCR in urine and serum samples following extraction by various methods.

Lanes 1-6 (amplification of DNA extracted by protocol A, B, C, D, E, F); lanes 8-11 (amplification of external standard: 0.01, 0.1, 1 and 10 ng of pBKV).

values of DNA recovery percentage: 76.5%, 72.5%, 75.5%, and 76.5%, respectively. Considering DNA purity, for urine samples, protocol E was superior to the other five extraction methods, with a 260/280 nm-absorbance ratio equal to 1.58; similar values was obtained by protocols C and D, both exhibiting a ratio equal to 1.57. On the contrary, the lowest ratio (1.19) was obtained with protocol F. Ratios obtained by protocols A and B were 1.54 and 1.45, respectively. In serum samples, protocol D was superior to other methods, with a ratio equal to 1.73. The lowest values were obtained with protocol C (0.69) and F (0.95). Other protocols exhibited intermediate results (protocol A, 1.67; protocol B, 1.63; protocol E, 1.53).

The presence of inhibitors was overcome by all DNA extraction procedures in urine samples, as evidenced by semiquantitative PCR amplification of the eluted DNA. In serum samples, extraction protocols C and F did not completely overcome the presence of inhibitors, with a percentage of inhibition of 18% (0.8 ng semiquantitative results/ 1 ng amplified) and 14% (0.86 ng semiquantitative results/ 1 ng amplified), respectively (Tables 1 and 2, Figure 1).

## DISCUSSION

Although many different extraction procedures for PCR have been described, there have been relatively few studies comparing methods. The aim of this study was to compare six different DNA extraction procedures from urine and serum samples for subsequent PCR analysis. The six protocols here evaluated were designed to fulfill the following criteria:

- 1) sensitive, reproducible, rapid, and simple, requiring no specialized equipment or specific knowledge of biochemistry, thus allowing nucleic acids purification from urine and serum sample in a routine setting;
- 2) extracted DNA should be sufficiently pure to allow enzymatic modifications;
- 3) low risks for operators;

TABLE 3 - Summary of reported methods to overcome specific PCR inhibitors.

PCR inhibitor	Modification of PCR to overcome inhibition	Reference
Phenol	Use of <i>Tth</i> polymerase	Katcher and Schwartz 1994
Heparin	Incubation of nucleic acid sample with heparinase I	Izraeli <i>et al.</i> , 1991
Denaturated albumin	NaOH denaturation or octanoate thermoprotection as pretreatment	Vandenvelde <i>et al.</i> , 1993
Urine	Ultrafiltration with Millipore cups	Khan <i>et al.</i> , 1991
Unknown	Prolonged heat soaking at 94 °C	Ruano <i>et al.</i> , 1992
Acidic polysaccharides	Addition of Tween 20, dimethyl sulfoxide, or polyethylene glycol	Demeke and Adams 1992
Glove powder	Use a powderfree gloves or gloves washing prior to handling of tubes	de Lomas <i>et al.</i> , 1992
UV-treated mineral oil	Addition of 8-hydroxyquinoline	Gilgen <i>et al.</i> , 1995

4) limited likelihood of DNA transmission between samples.

In urine and serum samples, nucleic acids may be present in mammalian or prokaryotic cells as well as in viruses. Indeed, the first step of all DNA extraction procedures consists in a lysis (Chirgwin *et al.*, 1979; Firestein *et al.*, 1987; Hatefi and Hanstein, 1969; Vogelstein and Gillespie, 1979) in order to release nucleic acids which can then be purified. All the DNA extraction procedures involve an initial treatment with detergents to solubilize any cell components present, followed by a specific purification of the nucleic acid. The traditional extraction procedure of DNA from plasmids or clinical samples is based on phenol/chloroform extraction, followed by ethanol precipitation (protocol D) (Sambrook *et al.*, 1989). This method is still widely used in many research and diagnostic laboratories. Although the reagent cost is lower compared with the commercial nucleic acid extraction kit, this procedure requires the use of hazardous chemicals. This method would be expected to reduce the concentration of residual proteins and membrane components potentially inhibiting the enzymatic activity of *Taq* polymerase. In protocols A and B, samples were boiled (and not extracted with organic solvents), while in protocol F residual proteins were eliminated by proteolytic activity of proteinase K which digests histones that would otherwise remain strongly bound to DNA (Higuchi, 1989). The silica particle adsorption method (protocol E) and the lysis methods (protocol C) did not remove any residual proteins present in the samples.

In this study, we included a commercially available kit (Extragen, protocol A), recommended for the extraction of nucleic acids from acellular samples. Since the procedural steps in the lysis, proteolytic and phenol/chloroform methods are commonly not uniform among laboratories, we considered a procedure which could be readily standardized and made accessible to diagnostic laboratories. This method is designed to extract both DNA and RNA with yield and purity similar to those obtained by the phenol/chloroform method, but without using hazardous chemicals. Although all six extraction procedures display some advantages, the yield and purity of recovered DNA varied widely.

The highest yield of DNA was obtained by the protocol D (phenol/chloroform extraction procedure) in urine samples and protocol E (silica gel extraction procedure) in serum samples. Regarding DNA purity, protocol E was superior for urine samples and protocol D for serum samples. Although a recovery percentage of 87% by protocol F, it did not remove many proteins and polysaccharides potentially interfering with subsequent amplification. All extraction protocols resulted efficient regarding the absence of inhibitory substances in urine samples. On the contrary, protocols C and F did not overcome inhibition of the enzymatic activity of *Taq* polymerase in serum sample, with percentages of inhibition of 18% (0.8 ng semiquantitative results/ 1 ng amplified) and 14% (0.86 ng semiquantitative results/ 1 ng amplified), respectively (Tables 1 and 2, Figure 1).

In terms of costs and duration, extraction protocols B, C, D, E and F were comparable. Commercial protocol A was the most expensive, still DNA purity resulted similar to those obtained with protocols C, D and E for urine samples (Table 1) and with protocols B and D for serum samples (Table 2).

Over the last few years, since PCR amplification technology has been applied for diagnostics, many practical modifications have been suggested to overcome PCR inhibition. Table 3 summarizes the principal PCR inhibitors and the corresponding methods recommended for improving amplification results. In addition to the presence of inhibitors, improper handling and storage conditions could also reduce the PCR signal (Klein *et al.*, 1997).

The results of this study indicate that the occasional failure to detect small amounts of BKV sequences in serum samples may be due to the presence of factors inhibiting PCR amplification and that it is necessary to remove such factors from sera prior to PCR amplification.

In conclusion, our results demonstrate that phenol/chloroform and silica gel extraction procedures are the most efficient in terms of yield and purity of DNA.

Generalizations from the present study may be limited to urine and serum samples but our results could provide useful guidelines to develop protocols for other types of biological samples.

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