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SHORT COMMUNICATION

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Improved DNA typing of human urine by adding EDTA

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Abstract The effect of different EDTA concentrations on the DNA content of urine samples was examined and compared to untreated urine at various storage temperatures and times. The results indicate that adding EDTA increases the DNA stability for long time storage especially at low temperatures.

Key words Urine · EDTA · Storage conditions · DNA typing · Forensic

Introduction

In human urine, the DNA is contained in epithelial cells such as renal tubular, transitional urothelial, squamous cells and leukocytes. The extractable amount of DNA from urine samples depends on the extraction methodology (Pötsch et al. 1992), on the gender (Prescott et al. 1966), storage conditions (Prinz et al. 1993; Tsongalis et al. 1996), the extent of bacterial contamination as well as the release of nucleases from hydrolysed cells (Medintz et al. 1994). Since urine samples are of forensic interest for various reasons such as individualisation, DNA typing should be possible even after long term storage. With this aim the effect of EDTA on the DNA yield was tested by adding EDTA to urine samples and subsequent storage at different temperatures.

Material and methods

Freshly taken urine samples (300 ml) from 5 females and 5 males were agitated thoroughly and divided into 6 × 50 ml aliquots per individual. One third contained no EDTA, the other third contained EDTA at a final concentration of 40 mM EDTA. One of each third was stored at room temperature (r.t.) and –20° C.

The pellet from 1.5 ml urine was microscopically investigated without staining (× 400) using a Zeiss Axiola microscope. Since female urine usually contains a higher number of nucleated cells caused by a higher number of excreted leukocytes (Prescott et al. 1966) 1.5 ml from females and 6 ml from males were used for DNA extraction. DNA was extracted using the Chelex extraction protocol (Walsh et al. 1991) and quantified using the slot-blot technique (probe D17Z1, Gibco BRL, Waye et al. 1989). PCR amplification was performed with the Hum THO1 STR system according to Edwards et al. (1991) using 2 ng of template DNA. The alleles were separated on a horizontal polyacrylamide gel (6.8% T, 0.9% C, piperazine diacrylamide as crosslinker, 81 mM tris-formate, 28 mM cyclohexamineethane sulfonic acid).

Results and discussion

Microscopical examination of fresh urine revealed besides a few calcium oxalate crystals, a higher number of nucleated cells for females and a lower for males. After 32 days of storage an increase of calcium oxalate crystals and of bacterial and fungal contamination could be observed.

In the presence of EDTA a reduction of the contamination could be seen. In addition no crystals were noticed. This effect can be explained by binding of calcium via EDTA and therefore preventing further degradative effects (Iwasaki et al. 1997).

The amount of extractable DNA ranged from 53 ng to 200 ng/ml for females and from 3 ng to 50 ng/ml for males and one male sample contained 500 ng/ml. The standard deviation was s = 4.55 for females and s = 17.58 for males.

The relative loss of DNA versus storage time and temperature was identical for both genders. Therefore only the results for DNA from female urine samples are illustrated. Figure 1 shows the DNA yield plotted as a function of time at different storage temperatures (r.t. and –20° C) and EDTA concentrations (0 and 40 mM). Adding EDTA to a urine sample increased the DNA yield at both storage temperatures. On the other hand reducing temperature increased the DNA yield for both EDTA concentrations.

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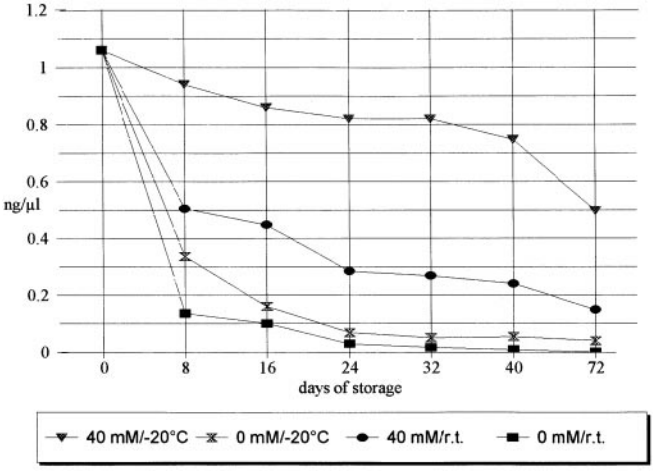

Fig. 1 Average DNA yield from 1.5 ml female urine plotted as a function of time for different temperatures (r.t. and -20° C) and EDTA concentrations (0 and 40 mM)

Furthermore the effect of adding EDTA is stronger in comparison to temperature reduction, because the DNA yields of the 40 mM EDTA samples stored at r.t. were higher than samples without EDTA stored at –20° C. Therefore the combination 40 mM EDTA and –20° C storage combination gives the best results and reduces the drastical drop of DNA yield in the first week. DNA typing could be carried out with all samples that contained 2ng of template DNA.

As already known for DNA degrading enzymes (Fibi et al. 1991), it is shown that EDTA has a stabilising effect on DNA in urine. For long time storage of urine samples a temperature of –20° C and the addition of 40 mM EDTA is recommended.

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