qPCR with mRNA isolated from urothelial cells from urine

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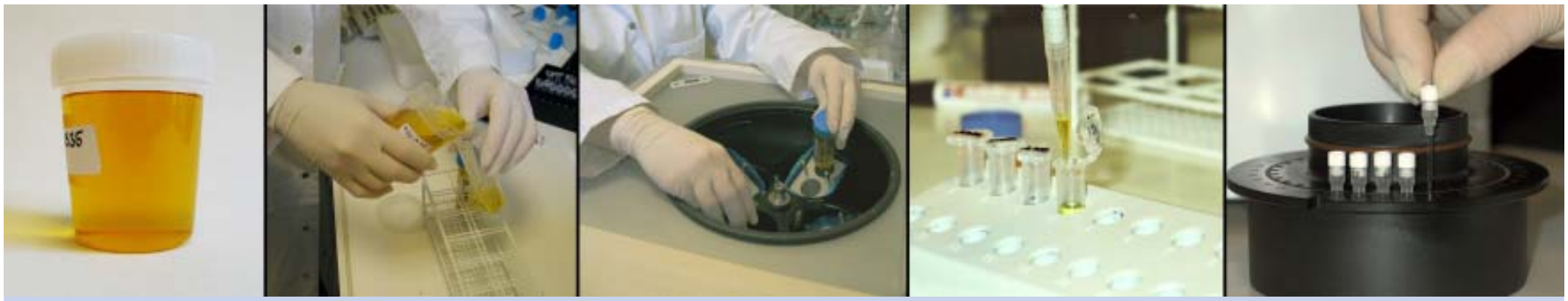

Fig. 1: The cells that are included in a urine sample are first collected from the sample by centrifugation. The pellet is carried over into the lysis buffer of the different isolation kits and frozen at -20°C until the sample is sent to our laboratory. The RNA isolation was done corresponding to the manufacturers' instruction. Afterwards, the Real-Time PCR measurement was performed on a LightCycler System.

Objectives

Bladder cancer in chemical workers is an occupational disease associated with previous exposure to aromatic amines. Currently, urine-based markers used for screening of high-risk collectives are not of high sensitivity. To detect cancer at earlier stages more suitable non-invasive markers are necessary. Some promising new tumor markers are based on mRNA quantitation. The aim of this study was to establish and optimize a practical and efficient mRNA isolation method that allows applying qPCR-based assays with urothelial cells from urine.

Methods

Six different isolation methods on the basis of commercially available kits were compared using urine samples of healthy donors and a control RNA with known concentration. A situation was simulated comparable to sample collection in a clinical setting. Cells were collected from urine by centrifugation and transferred to a buffer according to the manufactures recommendations. After short (48 h) storage at -20°C the mRNA isolation was performed. In all tested assays mechanical disruption of the cells was identical. The six isolation methods differed by DNA removal step (DNase treatment or special DNA column), material of the RNA columns (differences between companies) and the ß-Mercaptoethanol content (Tab. 1). After isolation, extracted RNA was transcribed to cDNA and quantified on a LightCycler system using an adapted Taqman-based assay for ß-Actin (FDI, Malvern, PA, USA). The results were compared and the four most reliable kits were tested again with a RNA of known concentration as template. Possible DNA contamination was monitored with „RT-Minus-PCR" control runs.

Tab. 1: The six different isolation methods tested varied essentially in type of DNA removal and content of ß-Mercaptoethanol

	Invitrogen – Pure Link Micro to Mini Total RNA Purification System*	Qiagen – Rneasy Mini Kit*	Macherey & Nagel – Nucleo Spin RNA II*	Qiagen – Rneasy Plus Mini Kit*	Invitek – Spin Cell RNA Mini Kit	Invitek – Spin Cell RNA Mini Kit with Carrier Suspension
type of DNA removal	Dnase	Dnase	Dnase	DNA column	DNA column	DNA column
type of RNA isolation	Column	Column	Column	Column	Column	Column
with ß-Mercaptoethanol	yes	yes	yes	yes	no	no

Tab. 2: The RNA isolation efficiency from urine samples measured by ß-Actin values was evaluated by sorting the results into two groups: above 1.000 copies and above 10.000 copies of ß-Actin. Three methods showed acceptable results (> 70% of the samples above 10.000 ß-Actin copies)

	Invitrogen – Pure Link Micro to Mini Total RNA Purification System*	Qiagen – Rneasy Mini Kit*	Macherey & Nagel – Nucleo Spin RNA II*	Qiagen – Rneasy Plus Mini Kit*	Invitek – Spin Cell RNA Mini Kit	Invitek – Spin Cell RNA Mini Kit with Carrier Suspension
ß-Actin values above 1.000 copies	38%	100%	100%	100%	100%	100%
ß-Actin values above 10.000 copies	0%	50%	100%	33%	75%	88%

isolation method works reliable | isolation method works not reliable | *contains ß-Mercaptoethanol

Results

- A method for RNA isolation from urine could be established (Tab. 2).
- mRNA isolated from urine as a starting material is highly contaminated with genomic DNA.
- Better yield and purity of mRNA could be achieved with application of a special DNA binding column that selectively binds the DNA
- Glass fibre-based separation of DNA allows recovery and use of the DNA for other applications.
- A quantitative comparison showed that best results could be obtained with the Invitek kit (± carrier suspension), followed by Macherey & Nagel (Fig. 2).

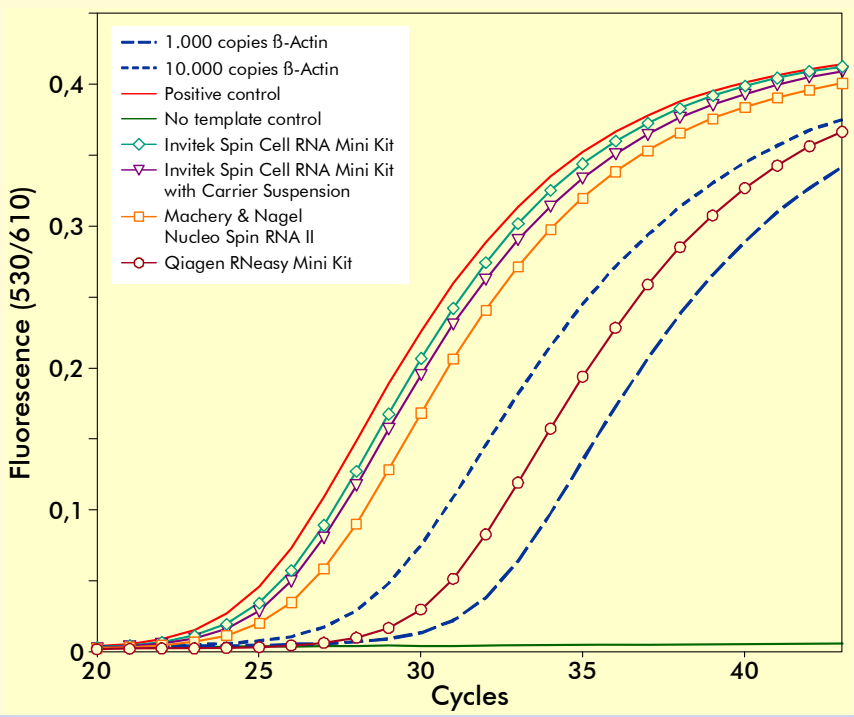

Fig. 2: RNA quantification based on standardized controls: Measurements were performed with the best four of the six evaluated kits. Depicted are 2 copy number controls (1.000 & 10.000 copies), positive and no template controls and the positive controls treated with the four different isolation methods.

Conclusions

Most of the six tested methods for mRNA isolation from urine are generally suitable for downstream qPCR applications.
However, good results can be obtained with a DNA column-based method (INVITEK, Berlin) that avoids DNase treatment. It excelled in two points: Reproducibility of yield even with very small amounts of starting material and reliability in the separation of DNA and mRNA.
Both properties are an absolute requirement for field studies where cell material is limited and frequently of varying quality. Because of the limited storage life and the toxicity buffer with ß-Mercaptoethanol should be avoided.

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