# DNA typeability in liquid urine and urine stains using AmpFlSTR SGM Plus

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## Abstract

**Purpose:** Urine specimens are usually collected for biochemical and toxicological tests and for doping control. In forensic casework urine analyses are performed occasionally, however, the authors emphasize their importance in crime scene reconstruction. The objective of the research was to evaluate efficacy of AmpFlSTR SGM Plus typing of urine and urine stains which were subject to different temperature conditions.

**Material and methods:** Urine samples were collected from 10 female and 10 male volunteers. Liquid specimens were stored at room temperature (RT), 4°C and -20°C up to 28 days. Experimental stains were prepared by applying 3 ml urine on sterile cloth 30x30 cm, air-dried and stored at RT up to 360 days. The amount of DNA was estimated with use of slot-blot technique (Quantiblot Human DNA Quantitation Kit, Applera). DNA profiles were obtained using AmpFlSTR SGM Plus and 310 ABI Prism Genetic Analyzer (Applera). Typing of a experimental sample was considered successful when the full profile was obtained matching that of a reference sample.

**Results:** Significant differences in DNA yield were noted between female and male urine samples. No differences between the extraction methods were found in regard to DNA yield and typeability rate. Different typeability rates were recorded for liquid urine and urine stains depending on storage temperature.

**Conclusions:** Liquid urine samples and urine stains can be considered as a potential source of DNA in disputable specimen individualization and in forensic casework using the fluorescent multiplex PCR system AmpFlSTR SGM Plus.

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## Introduction

Normal human urine specimens normally contain low numbers (up to 400 cells/ml) of epithelial cells (i.e. renal tubular, transitional urothelial, and squamous) [1]. Commonly, urine samples are collected for biochemical and toxicological tests and for doping control. In these circumstances, assessment of sample origin is unnecessary, unless sample switching or handling are suspected. In forensic casework urine analyses are performed occasionally, particularly in sexual assaults, therefore identification and individualisation of urine stains and samples does not pose a medico-legal concern unlike bloodstains, saliva or sperm [2], however, the authors emphasize their importance in crime scene reconstruction. The objective of the research was to evaluate efficacy of AmpFlSTR SGM Plus typing of urine and urine stains subject to different temperature conditions.

## Material and methods

Urine samples were collected from healthy volunteers (10 females and 10 males). Liquid specimens were stored at room temperature (RT), 4°C and -20°C up to 28 days. In order to collect specimens for DNA extraction the latter samples were freeze/thawed every seven-days of incubation. Reference specimens were frozen once after the collection and thawed on the day 28. Experimental stains (n=20) were prepared by applying 3 ml urine on sterile cloth 30x30 cm, air-dried and stored at RT up to 360 days. Bloodstains collected from the same subjects served as reference. Urine samples of 1 ml volume were centrifuged at 13,600 x G for 5min. The supernatant was aspirated leaving 50 μl sediment. DNA samples were extracted from the liquid specimens and 2.0x2.0 cm stain cuts using Chelex 100 [3] and organic procedure [4]. The amount of DNA was

*Table 1*. **The typeable rates (%) of liquid urine samples using AmpFlSTR SGM Plus depending on storage temperature**

| Storage period | **Male** (n=10) | | **Female** (n=10) | |
|---|---|---|---|---|
| | Extraction | | | |
| | organic | chelex | organic | chelex |
| Fresh | 100 | 100 | 100 | 100 |
| RT (1 day) | 100 | 100 | 100 | 100 |
| RT (7 days) | 70 | 70 | 80 | 80 |
| RT (14 days) | 40 | 30 | 50 | 50 |
| RT (21 days) | 20 | 10 | 30 | 20 |
| RT (28 days) | 10 | 10 | 10 | 10 |
| 4°C (1 day) | 100 | 100 | 100 | 100 |
| 4°C (7 days) | 100 | 100 | 100 | 100 |
| 4°C (14 days) | 60 | 50 | 70 | 70 |
| 4°C (21 days) | 40 | 30 | 50 | 40 |
| 4°C (28 days) | 30 | 20 | 30 | 20 |
| -20°C (1 day) | 100 | 100 | 100 | 100 |
| -20°C (7 days) | 100 | 100 | 100 | 100 |
| -20°C (14 days) | 100 | 100 | 100 | 100 |
| -20°C (21 days) | 90 | 70 | 90 | 90 |
| -20°C (28 days) | 80 | 70 | 90 | 80 |

*Table 2*. **The typeable rates (%) of urine stains using AmpFlSTR SGM Plus depending on storage temperature**

| Storage period | **Male** (n=10) | | **Female** (n=10) | |
|---|---|---|---|---|
| | Extraction | | | |
| | organic | chelex | organic | chelex |
| RT (1 day) | 90 | 90 | 90 | 90 |
| RT (30 days) | 90 | 90 | 90 | 90 |
| RT (60 days) | 90 | 90 | 90 | 90 |
| RT (90 days) | 80 | 80 | 90 | 90 |
| RT (180 days) | 50 | 50 | 70 | 60 |
| RT (360 days) | 30 | 20 | 40 | 30 |

estimated with use of slot-blot technique (Quantiblot Human DNA Quantitation Kit, Applera). 1 ng target DNA was amplified using GeneAmp PCR System 9700 (Applera) according to the manufacturer's instructions (AmpFlSTR SGM Plus PCR Amplification Kit: User's Manual, Applera). Genotyping was performed in 310 ABI Prism Genetic Analyzer using GeneScan Analysis v3.1.2 and Genotyper v2.5 software. The typing of an experimental sample was considered successful when the full profile was obtained matching that of a reference sample.

**Statistical data analysis**

All obtained results were statistically analysed and expressed considering average measurement error (SEM). Statistical significance of all differences in respective results was assessed using ANOVA. The level of significance was 0.05. All data were standardised for each series.

## Results and discussion

Since urine contains minute amounts of nucleated epithelial cells and leucocytes, concentration of urine samples and cell sedimentation prior to DNA extraction is essential to efficient genotyping. The yield of DNA extracted from female and male liquid urine samples was 50-230 ng and 10-65 ng, respectively. The yield of DNA extracted from female and male urine stains was 1.5-10 ng and 0.3-3.5 ng, respectively. The differences were statistically significant ($p<0.05$). No differences between the extraction methods were found in regard to DNA yield and profile typeability rate. Similar results were obtained by Vu et al. [5], while Dimo-Simonin et al. [6] reported substantial differences in DNA yield between chelex and organic extraction. According to Prinz et al. [7] storage of 20 ml urine for 6 months at 4°C resulted in decrease of isolated DNA from 20-40 ng to 1-2 ng and from 400-800 ng to 10-20 ng for males and females, respectively.

The obtained typeability rates are summarized in *Tab. 1* and *2*. All fresh liquid samples were easily typeable with AmpFlSTR SGM Plus kit. For urine specimens stored at room temperature for 14 days the rate of typeable profiles decreased to 50% and below, due to absence of larger amplicons, most likely caused by DNA degradation. According to Schmitt et al. inconclusive results may result from allele drop-out due to small numbers of cells in urine specimens [8]. Other authors [7, 9, 10] found that removal of contaminants, including bacteria, from urine samples improves efficacy of DNA amplification. Liquid specimens stored at -20°C up to 28 days produced 70-90% typeability depending on extraction method and donor's sex. According to van der Hel et al. 33% of urine specimens submitted to long-term storage at -20°C yielded high-molecular weight DNA [11]. Also Dino-Simonin and Brandt-Casadevall [6] claimed that freezing is the best method of urine storage. In our material, following three freeze/thaw cycles negative effect on DNA quality was noted. Other authors indicated that repeated freeze/thaw cycles result in lysis of the urether epithelial cells in urine specimens facilitating release of nuclear DNA and its hydrolysis by endogenous nucleolytic enzymes which consequently diminishes the possible source of DNA [12,13]. For experimental urine stains stored up to 60 days the typeability rate was 90%. After 360 days typeability rate decreased to 20-40% depending on extraction method and donor's sex.

We conclude that liquid urine samples and urine stains can be considered as a potential source of DNA in disputable specimen individualization and in forensic casework using the fluorescent multiplex PCR system AmpFlSTR SGM Plus.

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