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Development of a dual test procedure for DNA typing and methamphetamine detection using a trace amount of stimulant-containing blood

Toshiaki Irii [a,b,*], Kyoko Maebashi [a], Kenji Fukui [a], Ryoko Sohma [a], Sari Matsumoto [a], Shojiro Takasu [a], Kimiharu Iwadate [a]

[a] Department of Forensic Medicine, The Jikei University School of Medicine, 3-25-8, Nishi-shimbashi, Minato-ku, Tokyo 105-8461, Japan
[b] Criminal Investigation Laboratory, Metropolitan Police Department, 3-35-21, Shakujiidai, Nerima-ku, Tokyo 177-0045, Japan

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ABSTRACT

Investigation of drug-related crimes, such as violation of the Stimulant Drug Control Law, requires identifying the used drug (mainly stimulant drugs, methamphetamine hydrochloride) from a drug solution and the DNA type of the drug user from a trace of blood left in the syringe used to inject the drug. In current standard test procedures, DNA typing and methamphetamine detection are performed as independent tests that use two separate portions of a precious sample. The sample can be entirely used up by either analysis. Therefore, we developed a new procedure involving partial lysis of a stimulant-containing blood sample followed by separation of the lysate into a precipitate for DNA typing and a liquid-phase fraction for methamphetamine detection. The method enables these two tests to be run in parallel using a single portion of sample. Samples were prepared by adding methamphetamine hydrochloride water solution to blood. Samples were lysed with Proteinase K in PBS at 56 °C for 20 min, cooled at −20 °C after adding methanol, and then centrifuged at 15,000 rpm. Based on the biopolymer-precipitating ability of alcohol, the precipitate was used for DNA typing and the liquid-phase fraction for methamphetamine detection. For DNA typing, the precipitate was dissolved and DNA was extracted, quantified, and subjected to STR analysis using the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit. For methamphetamine detection, the liquid-phase fraction was evaporated with N_2 gas after adding 20 μL acetic acid and passed through an extraction column; the substances captured in the column were eluted with a solvent, derivatized, and quantitatively detected using gas chromatograph/mass spectrometry. This method was simple and could be completed in approximately 2 h. Both DNA typing and methamphetamine detection were possible, which suggests that this method may be valuable for use in criminal investigations.

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1. Introduction

In Japan, ⩾10,000 people are arrested each year for violating the Stimulant Drug Control Law, representing >80% of all drug cases [1]. Major methods for using stimulant drugs in Japan include intravenous injection, absorption, and ingestion [2]. When investigating drug-related crimes involving intravenous injection of a stimulant, the drug can be identified from the residual solution in the syringe, and the drug user's DNA type can be determined by analyzing trace amounts of blood in the syringe after injecting the drug.

In current standard test procedures, DNA typing and drug analysis are independently performed. In the personal identity by DNA analysis, the personal difference with the target person was performed by examinations for short tandem repeat (STR) analysis with various multiplex STR amplification kit [3–11]. The result of the STR analysis can also clarify that trace blood in syringe was from a single person or multi-person because of syringe sharing [12]. The proof of using the stimulant by the recognition of stimulant detection was performed using thin-layer chromatography (TLC), gas chromatography–mass spectrometry (GC/MS), liquid chromatography–mass spectrometry (LC/MS) and high performance liquid chromatography (HPLC) [13–15].

* Corresponding author at: Department of Forensic Medicine, The Jikei University School of Medicine, 3-25-8, Nishi-shimbashi, Minato-ku, Tokyo 105-8461, Japan.
E-mail address: irii@jikei.ac.jp (T. Irii).

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In addition, these analysis methods have been performed for not only investigating drug-related crimes but also investigating anti-doping [16,17].

In DNA typing and drug analysis, each test procedure requires two separate portions of the sample. In cases where sample volumes are extremely small, a sample may be completely used up by only one of these tests. In addition, the formation of solid blood clots in a syringe makes it difficult to collect a test sample by just washing with distilled water, and the clots should be dissolved prior to analysis (Fig. 1).

To overcome these problems, we developed and tested a new procedure involving partial lysis of a stimulant-containing blood sample followed by separation of the lysate into a precipitate for DNA typing and a liquid-phase fraction for drug testing. In other words, this method divides washings including blood into components derived from a blood corpuscle and liquid. Nakazono et al. have reported the separation of steeping urine stain using a filtration device [18]. This method may aid in separating a part of the components derived from the blood corpuscle and liquid. However, we used a different approach because blood cells in our sample were lysed using protease.

We selected methanol for high volatility so that the organic solvent could be removed from the liquid-phase fraction for methamphetamine detection. According to the general method of ethanol precipitation, we tried using methanol instead of ethanol because methanol had the biopolymer-precipitating ability as it was an alcohol and polar solvent. In addition, in treatment with Proteinase K, PBS contained salts such as NaCl, KH_2PO_4, and Na_2HPO_4. Although the biopolymer-precipitating ability of methanol was inferior to ethanol, isopropanol, and polyethylene glycol, we considered that the amount of DNA yield from 1 μL blood using this method was sufficient for STR analysis.

Thus, on the basis of the biopolymer-precipitating ability of alcohol, the precipitate was used for DNA typing and the liquid-phase fraction for methamphetamine detection. This method allows the two tests to be simultaneously run in a simple manner using only a single portion of sample. In this study, we examined the validity of this method by comparing the control sample without this method and limits of sensitivity. In addition, this method was performed on syringe samples as an application of forensic samples.

2. Material and methods

2.1. Test samples

Blood samples were provided by eight adult volunteers (four males, four females; mean age = 32.6 ± 6.9 years) who were not taking any medications. This study was approved by the Ethics Committee of The Jikei University School of Medicine for Biomedical Research (25–112). All volunteers provided written informed consent.

Fig. 1. Blood clots into a syringe.

The stimulant used in this study was methamphetamine hydrochloride (MA), (Sumitomo Dainippon Pharma Co., Ltd., Tokyo, Japan), which is the primary ingredient of a drug used illegally in Japan [14]. All test samples were prepared by adding 1 μL MA water solution to 1 μL blood that was either undiluted or diluted using phosphate-buffered saline (PBS; pH 7.2; Thermo Fisher Scientific Inc., Waltham, MA). The concentration of MA water solution and dilution ratio of blood for each test samples are shown in Table 1.

2.2. Sample separation

According to the general method of ethanol precipitation, samples were lysed with Proteinase K (20 mg/ml; QIAGEN, Hilden, Germany) in PBS at 56 °C for 20 min, cooled at −20 °C for 1 h after adding methanol, and then centrifuged at 15,000 rpm (Fig. 2). Using this method, the lysate was separated into a blood cell-derived fraction as a precipitate for DNA typing and a liquid-phase fraction for methamphetamine detection. In addition, the enzyme protein of Proteinase K in the lysate was collected as a part of the precipitate by denaturation and precipitation using methanol.

2.3. DNA typing

From the precipitate, DNA was extracted using a QIAamp DNA Investigator Kit (QIAGEN) according to the manufacturer's protocol [19]. Final elution was performed by adding 50 μL buffer ATE to ensure that the membrane was completely covered.

DNA was quantified by real-time PCR using the D17Z1 locus, which generated an amplicon of 207 bp [20]. Real-time PCR was performed using an Applied Biosystems StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific Inc.). The lower limit was set at 0.001 ng/μL.

DNA typing was performed by STR analysis using an AmpFLSTR® Identifiler® Plus PCR Amplification Kit (Thermo Fisher Scientific Inc.). The procedure was followed as per a manual of kit, using 25 μL of the PCR reaction mix and 1 ng of the DNA template, 28 cycles were run [21]. The minimum quantity of DNA template that full profiles were detected in 28 cycles was 0.125–0.25 ng [5,21,22]. The maximum volume of the template DNA solution which could be added in a PCR reaction mix was 10 μL; therefore, if the concentration of the provided DNA solution did not reach 0.1 ng/μL, a DNA solution of 10 μL was used for PCR amplification. Electrophoresis of PCR products was performed using an ABI PRISM 3130 XL Genetic Analyzer (Thermo Fisher Scientific Inc.). DNA type was determined with the minimum peak height set at 150 RFU.

Table 1
The concentration of MA water solution and dilution ratio of blood for preparing each test sample.

Sample name	Concentration of MA water solution (ng/μL)	Dilution ratio of blood	The subsection number using samples
Sample 1	500	Undiluted	2.5.1–2.5.5.
Sample 2	500	×5	2.5.2.
Sample 3	100	Undiluted	2.5.3. and 2.5.4.
Sample 4	100	×5	2.5.5.
Sample 5	50	×10	2.5.5.
Sample 6	25	×20	2.5.5.

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