Korean J Leg Med 2016;40:78-82
http://dx.doi.org/10.7580/kjlm.2016.40.3.78

Original Article

Factors Affecting DNA Yields from Serum and Plasma Samples Used for Personal Identification Testing

Ji Hyun Lee[1]*, Boram Kim[1]*, Sohee Cho[2], Hee Jin Seo[1], Soong Deok Lee[1,2]

[1]*Department of Forensic Medicine, Seoul National University College of Medicine, Seoul, Korea,* [2]*Institute of Forensic Science, Seoul National University College of Medicine, Seoul, Korea*

*These authors contributed equally to this work.

Received: July 26, 2016
Revised: August 22, 2016
Accepted: August 26, 2016

Correspondence to
Soong Deok Lee
Department of Forensic Medicine, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-740-8359
Fax: +82-2-764-8340
E-mail: sdlee@snu.ac.kr

Serum or plasma is free of cellular components. As DNA is in the nucleus or mitochondria of a cell, it can be presumed that serum/plasma is DNA free. However, there are cases wherein serum/plasma is the only resource available for identification analysis, yet no sufficient data are available regarding whether reliable DNA testing can be applied to such cases, and what the influencing factors are when testing is a valid course of action. The aim of this study is to illustrate the factors that can be used in the genetic testing of serum/plasma when identifying an individual. The results showed that the concentration of serum DNA significantly increased over time in 4°C storage, and the DNA yields from samples stored in heparin tubes were overall higher than from samples stored in ethylenediaminetetraacetic acid tubes. We observed that the concentration of DNA in serum successfully matched 100% to the short tandem repeat data of blood DNA.

Key Words: Serum; Plasma; DNA fingerprinting; Identification

Introduction

It has been generally accepted that blood serum and plasma are free of cellular components. Although the precise quality and quantity of DNA needed for reliable identification analysis is unknown [1,2], the cell debris and molecules released by cells during apoptosis are known to provide sufficient amounts of DNA for genetic analysis [1,3–6].

In real-time situations, serum/plasma samples can play a crucial role in personal identification. For instance, if patient samples prepared for testing are mixed up in-hospital for any reason, the consequences can be critical. In such cases, the serum/plasma DNA can be extracted from the patient's sample and can be used to conduct a search for the identification of its rightful owner.

Materials and Methods

Experiments were divided into time-temperature groups for serum and plasma with anticoagulant agents.

pISSN 2383-5702
eISSN 2383-5710

Blood was collected twice from 15 healthy individuals (10 men and 5 women), and once from another 14 healthy individuals (9 men and 5 women, excluding one subject who withdrew from the first experiment). The collection of samples for this study was conducted in accordance with the guidelines and approval of the Institutional Review Board of Seoul National University Hospital, Korea.

In order to set a parameter that affects the quantity and quality of DNA in a situation similar to that of a hospital, sample storage at room temperature and at 4°C was considered. Similarly, timeframe experiments were carried out assuming that the samples would be used within 48 hours, in accordance with the screening tests in a hospital, and that the results would be reported within 10 days. In the first experiment, samples stored for 0 hour, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 1 day, and 2 days were analyzed; in the second experiment, those stored for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days were analyzed. For the anticoagulant experiment, with heparin and ethylenediaminetetraacetic acid (EDTA), the samples were analyzed separately with storage time points of 0, 1, 3, and 7 hours. Blood samples were centrifuged at 3,000 ×g and dispensed into heparin and EDTA tubes at specific time points, taking great care not to mix the buffy coat or blood clots.

DNA was extracted from plasma and serum samples using a QIAamp Blood Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's recommendations. The quantity of DNA was determined using an Applied Biosystems Quantifiler Human DNA quantification Kit (Life Technologies, Carlsbad, CA, USA) on an Applied Biosystems 7000 sequence detection system (Life Technologies). The DNA was amplified using a AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA), and amplification was performed in a GeneAmp PCR 9700 thermocycler (Applied Biosystems) following the manufacturer's guidelines. The polymerase chain reaction (PCR) products were analyzed using a 3130XL Genetic Analyzer (Life Technologies).

Table 1. Amount of DNA recovered from donors (15 and 14) with time-temperature differences by two-way ANOVA

Time	RT (ng/μL)	4°C (ng/μL)	t	p-value
0 hr	0.1441±0.1480	0.1441±0.1480	0	>0.990
1 hr	0.1525±0.1228	0.1875±0.2687	0.0799	0.937
2 hr	0.1125±0.1136	0.0839±0.0723	0.0652	0.948
4 hr	0.1039±0.0778	0.2597±0.2171	0.3554	0.723
8 hr	0.1636±0.3502	0.2216±0.1060	0.1323	0.895
12 hr	0.1530±0.2022	0.3362±0.2755	0.4179	0.677
24 hr	0.1177±0.0682	0.9903±0.9097	1.991	0.049
48 hr	0.2321±0.1585	4.939±4.767	10.74	<0.001
0 day	0.2025±0.1938	0.2025±0.1938	0	>0.990
1 day	0.0552±0.0338	0.7881±1.099	0.8800	0.380
2 days	0.0639±0.0561	1.845±2.136	2.138	0.033
3 days	0.1391±0.1136	3.228±3.150	3.709	<0.001
4 days	0.1746±0.1018	2.961±3.026	3.345	0.001
5 days	0.3432±0.1755	3.030±3.250	3.226	0.001
6 days	0.5806±0.2739	3.674±5.452	3.714	<0.001
7 days	0.5105±0.2885	2.798±2.936	2.746	0.006
8 days	0.7600±0.3676	2.768±4.107	2.411	0.017
9 days	1.066±0.5695	2.144±3.483	1.295	0.196
10 days	1.444±0.6514	1.670±1.706	0.2717	0.786

Values are presented as mean±standard deviation.
RT, room temperature.

Results

Quantitative results are presented as the mean±standard deviation. Differences between groups were tested using a one-way analysis of variance (ANOVA); for repeated measures, a two-tailed unpaired t test (p-value) was used. Data analysis was performed using GraphPad Prism V6.0 software (Graphpad Software, San Diego, CA, USA) and Microsoft Excel 2007. A $p<0.05$ was considered statistically significant.

There was no statistically significant difference among the median cell-free DNA yields from blood samples stored at room temperature for various periods ($p>0.05$, paired t test). However, in the case of samples stored at 4°C, we found a significant difference between the amount of cell-free DNA extracted immediately and that of cell-free DNA extracted 8, 12, 24, and 48 hours after venesection ($p=0.010$, $p=0.004$, $p=0.003$, $p=0.001$, paired t test).

Data from two-way ANOVA of the amount of DNA extracted from serum are presented in Table 1. A comparison analysis of the DNA yields by time points, from serum samples stored at room temperature and 4°C is demonstrated in Fig. 1A and B. The differences were observed depending on the time point, revealing an overall trend of the amount of DNA increasing over

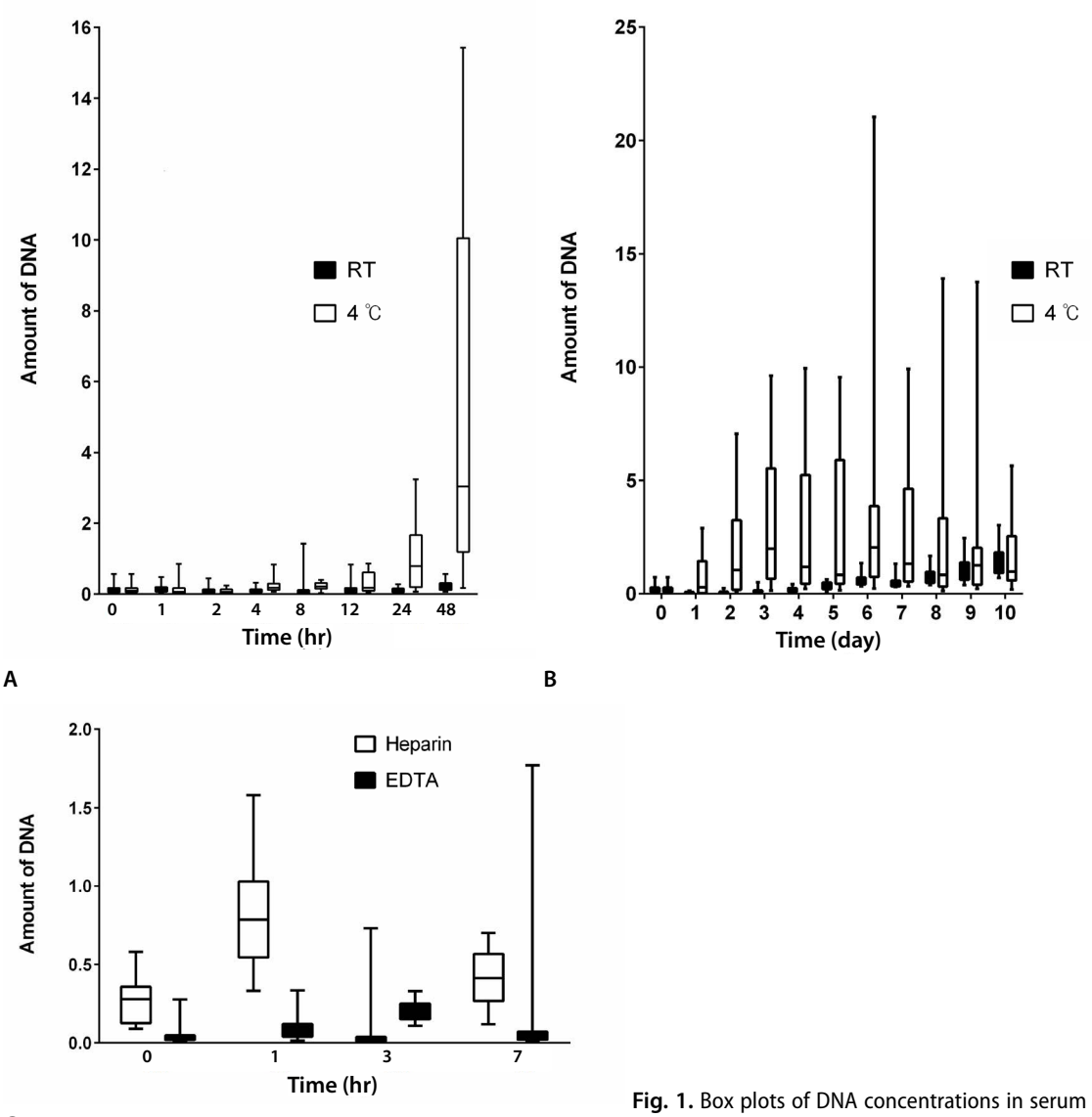


Fig. 1. Box plots of DNA concentrations in serum (**A, B**) and plasma (**C**). RT, room temperature; EDTA, ethylenediaminetetraacetic acid.

http://dx.doi.org/10.7580/kjlm.2016.40.3.78

time with respect to the first experiment with serum. The amount of DNA in the serum appeared to be significantly higher for the sample stored at 4°C for 48-hour in the first experiment, and that stored for 3–8 days in the second serum experiment. Here, we observed that the concentration of DNA in serum samples increases during storage at 4°C. In the first experiment, the DNA yield at 48 hours was approximately twice as high as that at room temperature, and approximately 5 times higher than that for samples stored at 4°C for 24 hours; however, we found little or no difference between DNA yields from samples stored for 1 or 2 days in the second experiment. This could be due to the frequent opening and closing of the refrigerator that resulted in falsely degraded DNA, or it could be associated with the physical condition of each individual who participated in the experiment. In other words, refrigeration is an appropriate storage method for protein-containing samples, such as serum; however, refrigeration may not be suitable for storing samples for DNA extraction; repeated changes in temperature conditions may interfere with DNA-dependent test results.

With regard to the effects of time and anticoagulants (heparin, EDTA) on plasma DNA, DNA yields from the plasma samples stored in heparin tubes were higher than from those stored in EDTA tubes, denoted by a two-way ANOVA, except for the time point of 3 hours after blood collection (Table 2, Fig. 1C). According to Lam et al. [7], no statistically significant difference in DNA yields is associated with anticoagulants for up to 6 hours of storage. However, in the present study, a significant difference in DNA yields was noted with regard to EDTA and heparin independent of time (p=0.013), despite the expectation that the DNA yields from samples stored in heparin tubes would increase over time. Therefore, for obtaining DNA samples, the use of heparin tubes is encouraged when collecting blood.

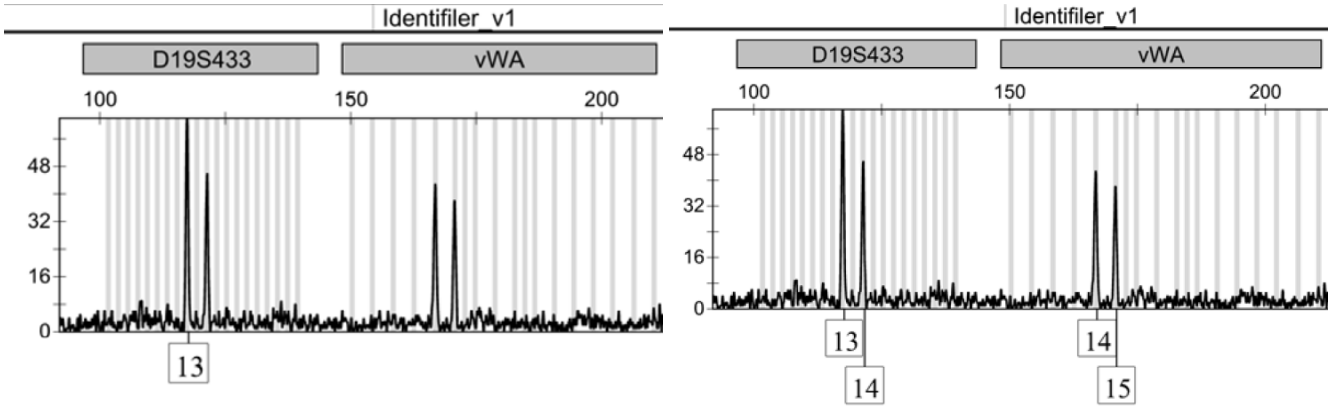
Table 2. Amount of DNA recovered from 15 donors with time-anticoagulant (heparin, EDTA) differences by two-way ANOVA

Time	Heparin (ng/μL)	EDTA (ng/μL)	t	p-value
0 hr	0.2672±0.1352	0.0448±0.0661	2.608	0.013
1 hr	0.8045±0.3414	0.0877±0.0828	8.406	<0.001
3 hr	0.0712±0.1836	0.2056±0.0621	1.576	0.123
7 hr	0.4122±0.1797	0.1632±0.4480	2.920	0.006

Values are presented as mean±standard deviation.
EDTA, ethylenediaminetetraacetic acid.

Discussion

In this study, we observed a trend of increase in DNA yields from blood serum and plasma samples with increasing storage period until a saturation point after a certain period, after which the quality of DNA began to degrade. For some samples, a drop-out phenomenon was observed. Within 10 days of storage, it was possible to obtain 100% matching results compared with the short tandem repeat data of blood DNA when the analytical threshold was lowered from 50 relative



A B

Fig. 2. Electropherograms of the amplified DNA from one sample. (**A**) Peak exceeding the 50-RFU threshold is present. (**B**) Peaks exceeding the 40-RFU threshold are present. RFU, relative fluorescence unit.

fluorescent units (RFU) to 40 RFU. Samples can be distinguished from the base-line peak by lowering the standards to 40 RFU (Fig. 2). Moreover, it should be possible to obtain results without changing the threshold if the number of PCR cycles is adjusted and the sample storage process is properly regulated [8]. However, it is uncertain whether the quality of DNA extracted after 10 days of storage is suitable for personal identification tests.

The results of this study are expected to be helpful in interpreting the effect of various factors that may influence the personal identification outcomes. Serum and plasma are targeted in various diagnostic fields and immunological tests, such as non-invasive tests for cancer patients and pregnant women, or in plastic surgery [9,10]. Human serum/plasma DNA is widely known to share the common characteristic of low copy number DNA, which can lead to frequent allele drop-out and drop-in occurrences. The results of this study are expected to aid in various types of diagnostic testing and herald serum/plasma as a newly discovered source for DNA, available for individual identification testing.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2014M3A9E1069989).

References

1. Lo YM, Tein MS, Lau TK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768-75.
2. Lee JH, Lee HY, Cho S, et al. DNA profiling via short tandem repeat analysis by using serum samples. Korean J Leg Med 2013;37:220-3.
3. Takayama T, Yamada S, Watanabe Y, et al. Origin of DNA in human serum and usefulness of serum as a material for DNA typing. Leg Med (Tokyo) 2001;3:109-13.
4. Honda H, Miharu N, Ohashi Y, et al. Fetal gender determination in early pregnancy through qualitative and quantitative analysis of fetal DNA in maternal serum. Hum Genet 2002;110:75-9.
5. Bischoff FZ, Sinacori MK, Dang DD, et al. Cell-free fetal DNA and intact fetal cells in maternal blood circulation: implications for first and second trimester non-invasive prenatal diagnosis. Hum Reprod Update 2002;8:493-500.
6. Allen RW, Pritchard JK. Resolution of a serum sample mix-up through the use of short tandem repeat DNA typing. Transfusion 2004;44:1750-4.
7. Lam NY, Rainer TH, Chiu RW, et al. EDTA is a better anticoagulant than heparin or citrate for delayed blood processing for plasma DNA analysis. Clin Chem 2004;50:256-7.
8. Ravard-Goulvestre C, Crainic K, Guillon F, et al. Successful extraction of human genomic DNA from serum and its application to forensic identification. J Forensic Sci 2004;49:60-3.
9. Barra GB, Santa Rita TH, Chianca CF, et al. Fetal male lineage determination by analysis of Y-chromosome STR haplotype in maternal plasma. Forensic Sci Int Genet 2015;15:105-10.
10. Sedlackova T, Repiska G, Celec P, et al. Fragmentation of DNA affects the accuracy of the DNA quantitation by the commonly used methods. Biol Proced Online 2013;15:5.

 http://dx.doi.org/10.7580/kjlm.2016.40.3.78