Typeability of AmpFlSTR SGM Plus loci in kidney, liver, spleen and pancreas tissue samples incubated in different environments

Niemcunowicz-Janica A[1], Pepiński W[1], Janica JR[2], Skawrońska M[1], Janica J[1], Koc-Żórawska E[1]*

[1] Department of Forensic Medicine, Medical University of Białystok, Poland
[2] Department of Radiology, Medical University of Białystok, Poland

Abstract

Purpose: The aim of the study was assessment of environmental effect on typeability of AmpFlSTR SGM Plus loci: D3S1358, VWA, D16S539, D2S1338, D81179, D21S11, D18S51, D19S433, TH01, FGA and gender marker amelogenin.

Material and methods: Kidney, liver, spleen and pancreas tissue specimens collected during autopsies of five persons aged 20-30 years were incubated at 21°C and 4°C in different environmental conditions. DNA was extracted by organic method from tissue samples collected in 7-day intervals and subsequently typed using AmpFlSTR SGM Plus PCR Amplification Kit and ABI 310.

Results: A fast decrease in typeability rate was seen in all tissue specimens incubated in peat soil and in sand. The specimens immersed in pond water and in salt water were partially typeable in all SGM Plus loci within 126 days. Increased air access and higher temperature during our experiments favoured desiccation and preservation of the material resulting in longer typeability of full SGM Plus.

Conclusion: Decomposed soft tissues are potential material for DNA typing.

Key words: forensic science, tissue decomposition, environmental conditions, DNA typing, AmpFlSTR SGM Plus.

* CORRESPONDING AUTHOR:
A. Niemcunowicz-Janica
Department of Forensic Medicine, Medical University of Białystok
ul. Waszyngtona 13, 15-230 Białystok, Poland
Tel: +48 85 7485950, Fax: +48 85 7485985

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Introduction

Automated fluorescence analysis of PCR amplified short tandem repeat systems (STRs) by capillary electrophoresis has gained a widespread usage in forensic medical practice [1-4]. An implementation of multiplex PCR kits and fluorescence based DNA detection allow increased sensitivity, unmatched accuracy and high throughput of samples for forensic casework analysis and paternity tests. STR alleles can be rapidly determined using commercially available kits. Typing of STR from highly degraded body is usually based on DNA extracted the from most resistant tissues, e.g., teeth, bones, hairs [5-9]. On the other hand, DNA extraction from soft tissue is easier and less time consuming. Organic extraction of DNA is reported by Takahashi et al. as a useful method in case of decomposed human tissue [10]. AmpFlSTR SGM Plus kit was validated as highly specific and sensitive for human DNA and suitable in typing of degraded samples [11].

The aim of the study was assessment of typeability of AmpFlSTR SGM loci in kidney, liver, spleen and pancreas specimens depending on different environmental conditions.

Material and methods

Kidney, liver, spleen and pancreas specimens were collected according to recommended anatomical body sections (abdomen) during autopsies of five persons aged 20-30 years with post mortem interval (PMI) limited to 14 hours. All the persons died due to hypothermia and early signs of body decomposition were prevented by storage in morgue refrigerator. Tissue specimens of dimensions 2×2×2 cm were incubated at 4°C and 21°C in closed 40 ml containers and at 21°C in closed 40 ml containers filled with sand, garden peat soil, pond water or salt water and at 21°C in open 40 ml containers. Five samples of each tissue were collected in 7-day intervals. DNA was extracted from 5 mg tissue by modified organic pro-

***Table 1*. Typeability of AmpFlSTR SGM Plus loci in kidney specimens**

Conditions	D3S1358	VWA	D16S539	D2S1338	XY	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
4ºC, closed container	91/119	84/105	70/98	63/84	105/126	56/84	70/98	56/84	98/119	91/119	63/91
21ºC, closed container	70/98	63/84	49/77	42/63	84/105	35/63	49/77	35/63	77/98	70/98	42/70
21ºC, open container	77/105	70/91	56/84	49/70	91/112	42/70	56/84	42/70	84/105	77/105	49/77
21ºC, salt water in closed container	42/84	35/77	28/70	21/49	56/105	42/77	28/70	14/49	49/98	42/77	21/56
21ºC, pond water in closed container	63/98	49/84	42/84	28/63	77/126	56/91	42/84	21/49	70/98	56/91	35/77
21ºC, sand in closed container	7/21	7/21	1/21	1/21	14/21	7/21	1/21	1/21	14/21	7/21	7/21
21ºC, peat soil in closed container	1/21	1/14	1/14	1/14	1/21	1/21	1/14	1/14	1/21	1/21	1/14

***Table 2*. Typeability of AmpFlSTR SGM Plus loci in liver specimens**

Conditions	D3S1358	VWA	D16S539	D2S1338	XY	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
4ºC, closed container	35/70	21/63	21/56	21/42	56/84	28/63	21/56	14/42	35/77	28/63	21/49
21ºC, closed container	21/54	7/35	7/35	1/35	21/56	14/35	7/35	7/35	21/56	14/42	7/35
21ºC, open container	28/49	14/56	14/56	14/49	28/77	21/56	14/56	14/42	28/63	21/49	14/49
21ºC, salt water in closed container	63/126	63/126	56/112	56/98	70/140	56/119	56/119	56/98	70/126	56/126	56/105
21ºC, pond water in closed container	70/126	63/126	56/112	49/98	70/133	56/126	56/119	49/98	70/126	56/126	56/105
21ºC, sand in closed container	14/21	14/21	7/21	7/21	7/21	14/21	7/21	7/21	14/21	7/21	7/21
21ºC, peat soil in closed container	14/21	14/21	14/21	7/14	14/21	14/21	7/21	7/14	14/21	7/21	7/21

cedure. The specimens were placed in 1.5 ml eppendorf tubes and incubated overnight at 56°C for 12 hrs in 0.5 ml digest buffer pH 7.5 (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS) with 0.3 mg/ml proteinase K (Sigma). Centrifuged pellets (Eppendorf, 16500 rpm, 1 min) were discarded and aspirated supernatants were transferred to fresh tubes containing 0.5 ml phenol-chloroform-isoamyl alcohol mix (Sigma). After centrifugation at 16500 rpm for 5 min (Eppendorf), resulting supernatants were transferred to fresh tubes. The latter step was repeated 2-3 times until the phenol phase became transparent. DNA preparations were concentrated and purified using QIAquick PCR Purification Kit (Qiagen). Reference DNA profiles were typed in fresh blood samples collected from respective corpses on autopsy. Recovered DNA was quantitated fluorometrically [12,13]. DNA quality was assessed by ethidium bromide 2% agarose gel electrophoresis. Ten polymorphic systems: D3S1358, VWA, D16S539, D2S1338, D81179, D21S11, D18S51, D19S433, TH01, FGA and gender marker amelogenin included in AmpFlSTR SGM Plus PCR Amplification Kit were amplified following the manufacturer's instructions (Applera, USA) with the exception, that the all reaction reagents were reduced proportionally so that volume of the reaction mix was 10 µl. Electrophoresis and genotyping were performed in ABI 310 Genetic Analyzer (Applera, USA) using Genescan v. 3.11 and Genotyper v2.5 software. As a threshold value a signal of 150 RFU was assumed.

Results

Extracted DNA yield ranged 0-5 ng. AmpFlSTR SGM Plus typeability limits for the tissues under study are presented in *Tab. 1*, *2*, *3*, *4*. First values denote time limits in days, when full AmpFlSTR SGM Plus profiles were typeable in all samples. Second values denote time limits in days, after which no AmpFlSTR SGM Plus profiles were seen for the set of 5 × 5 samples as a whole. In time spans between the two values partial profiles were observed.

***Table 3*. Typeability of AmpFlSTR SGM Plus loci in spleen specimens**

Conditions	D3S1358	vWA	D16S539	D2S1338	XY	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
4ºC, closed container	49/91	42/84	35/70	21/56	63/126	42/91	35/70	21/49	56/98	42/91	28/56
21ºC, closed container	28/70	21/63	14/49	14/42	42/105	21/70	14/49	7/14	35/77	21/70	7/35
21ºC, open container	35/77	28/70	21/56	21/49	49/112	28/77	21/56	14/21	42/84	28/74	14/42
21ºC, salt water in closed container	35/77	28/70	14/49	7/42	70/119	28/70	14/49	7/35	42/84	28/70	14/49
21ºC, pond water in closed container	49/84	42/77	35/70	21/42	56/105	42/84	35/70	21/42	49/98	42/84	21/42
21ºC, sand in closed container	14/21	14/21	7/21	7/21	7/21	14/21	7/21	7/21	14/21	7/21	7/21
21ºC, peat soil in closed container	14/21	14/21	14/21	7/14	14/21	14/21	7/21	7/14	14/21	7/21	7/21

***Table 4*. Typeability of AmpFlSTR SGM Plus loci in pancreas specimens**

Conditions	D3S1358	vWA	D16S539	D2S1338	XY	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
4ºC, closed container	63/105	49/98	42/84	42/77	77/126	56/98	42/84	42/70	63/105	56/98	42/84
21ºC, closed container	42/84	28/77	21/63	21/56	56/105	35/77	21/63	21/49	42/84	35/77	21/63
21ºC, open container	49/91	35/84	28/70	28/63	63/112	42/84	28/70	28/56	49/91	42/84	28/70
21ºC, salt water in closed container	28/70	21/56	14/49	7/35	42/91	28/63	14/49	7/35	28/84	28/63	14/35
21ºC, pond water in closed container	49/84	42/77	35/63	21/56	56/105	42/84	35/70	21/42	49/98	42/84	21/56
21ºC, sand in closed container	7/21	7/21	1/21	1/21	14/21	7/21	1/21	1/21	14/21	7/21	7/21
21ºC, peat soil in closed container	7/21	7/21	1/14	7/14	7/21	7/21	1/14	7/14	7/21	7/21	7/14

Discussion

The authors evaluated typeability of AmpFlSTR SGM Plus kit loci D3S1358, VWA, D16S539, D2S1338, D81179, D21S11, D18S51, D19S433, TH01, FGA and gender marker amelogenin in kidney, liver, spleen and pancreas specimens incubated at 21°C and 4°C in different environmental conditions in the interval of 7 to 140 days. In our experiment fast DNA degradation was observed in tissue material stored in peat soil and in sand, which may result from humus acid content, microbial action or acid pH [14-16]. On the other hand, increased air access and higher temperature during our experiments favoured desiccation and preservation of the material [17,18] resulting in the prolonged typeability of full SGM Plus profiles in specimens stored at 21ºC in open containers when compared to that in closed containers. Incubation of kidney specimens in closed containers at 21ºC resulted in partial DNA degradation after 35 days. Liver specimens immersed in pond water and in salt water were typeable in all SGM Plus loci within 49 days. Hoff-Olsen et al. reported typing of seven STRs in the liver of decomposed corpse recovered from a lake after 90 days and from a river after 17 days [19]. In our experiment storage of liver specimens in closed containers at 21ºC resulted in partial SGM Plus profiles after 7 days except D2S1338 which was typed in all samples only on first day of the experiment. Hoff-Olsen et al. typed three STRs in liver samples collected from a decomposed body recovered from a house after 27 days [19]. Piasecka-Pazik et al. [20] reported lack of longer alleles of AmpFlSTR Identifiler loci typed in liver samples after 7-day incubation. Spleen specimens incubated in different conditions of our experiment were partially typeable within 119 days. Pancreas specimens incubated in different conditions were partially typed within 126 days. The experimental model assumed in our study does not reflect a typical process of decomposition, but can be used for identification of fragmentary tissue samples from victims of airplane, train and car accidents recovered from water, and sand or soil [21,22].

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