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TECHNICAL NOTE

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Typeability of AmpFlSTR SGM Plus Loci in Brain and Thyroid Gland Tissue Samples Incubated in Different Environments*

ABSTRACT: Autolysis and putrefaction are crucial factors responsible for degradation of cells, tissues, and organs. Postmortem changes may assume different course depending on extrinsic and intrinsic conditions. 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. Brain and thyroid gland 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 kit and ABI 310. A fast decrease in typeability rate was seen in specimens incubated in peat soil and in sand. Brain tissue samples were typeable in all AmpFlSTR SGM Plus loci within 126 days of incubation at 4°C. Faster DNA degradation was recorded in thyroid gland specimens. In samples with negative genotyping results, no DNA was found by fluorometric quantitiation.

KEYWORDS: forensic science, tissue decomposition, environmental conditions, DNA typing, AmpFlSTR SGM Plus

Autolysis and putrefaction are crucial factors responsible for degradation of cells, tissues, and organs. Autolysis involves enzymatic degradation of cells or tissues, and putrefaction is caused by saprophytic microbial organisms. Postmortem (PM) changes may assume different course depending on extrinsic and intrinsic conditions including age and weight, antemortem diseases, and injuries (1). The exterior of a corpse and condition of internal organs are related with environmental conditions. Reports concerning effects of body decomposition on DNA quality were based on PM time estimated by pathologists using information from the police authorities, circumstances of death and autopsy findings (2,3). The aim of the study was assessment of typeability of AmpFlSTR SGM Plus PCR amplification kit (Applera, Foster City, CA) loci in brain and thyroid gland specimens depending on different environmental conditions.

Materials and Methods

Brain and thyroid gland specimens were collected during autopsies of five persons aged 20–30 years with postmortem interval (PMI) limited to 14 h according to recommended anatomical body sections (head and neck). This classification has rationale in practical needs resulting from forensic practice in cases of body dismemberment. All the persons died due to hypothermia, and early signs of body decomposition were prevented by storage in morgue refrigerator. The occipital lobe and left lobe thyroid tissue specimens of dimensions $2 \times 2 \times 2$ 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 procedure. The specimens were placed in 1.5 mL eppendorf tubes and incubated overnight at 56°C for 12 h 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-Aldrich, Poznań, Poland). Centrifuged pellets (Eppendorf [Cambridge, U.K.], 16,500 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 16,500 rpm for 5 min (Eppendorf), resulting supernatants were transferred to fresh tubes. The latter step was repeated two to three times until the phenol phase became transparent. DNA preparations were concentrated and purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Reference DNA profiles were typed in fresh blood samples collected from respective corpses on autopsy. Recovered DNA was quantitated fluorometrically (4,5). 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) with the exception that the all reaction reagent were reduced proportionally so that volume of the reaction mix was 10 μL. Electrophoresis and genotyping were performed in ABI310 Genetic Analyzer (Applera) using Genescan v.3.11 and Genotyper v2.5 software. As a threshold value a signal of 150 RFU was assumed.

Results and Discussion

Processes of autolysis and decomposition have always been a concern to forensic experts. Depending on environmental

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TABLE 1—*Typeability of AmpFlSTR SGM plus loci in brain tissue specimens.*

Conditions	D3S1358	VWA	D16S539	D2S1338	XY	D8S1179	D21S11	D18S51	D19S433	THO1	FGA
4°C, closed container	154*	154	133	126	154	154	126	126	154	133	126
	175†	161	154	154	182	175	154	154	168	161	154
21°C, closed container	147	140	126	126	154	147	126	126	154	140	126
	168	168	147	147	175	168	147	140	175	168	147
21°C, open container	154	147	126	126	154	154	126	126	154	133	126
	175	168	154	147	182	168	161	140	175	154	147
21°C, sand in closed container	14	14	14	14	21	14	14	14	21	14	14
	28	28	21	21	28	28	28	21	28	28	21
21°C, peat soil in closed container	14	14	14	14	21	21	21	14	21	21	14
	35	35	35	21	35	35	28	21	35	35	21
21°C, pond water in closed container	119	112	112	112	126	112	112	112	126	112	112
	133	133	119	119	140	133	133	119	140	133	119
21°C, salt water in closed container	126	126	119	119	126	126	119	112	126	119	119
	140	140	133	133	140	140	133	126	140	133	126

*The value indicate time limits, in which full AmpFlSTR SGM Plus profiles were typed.
†The value indicate time limits, after which no AmpFlSTR SGM Plus profiles were typed.

TABLE 2—*Typeability of AmpFlSTR SGM Plus loci in thyroid gland tissue specimens.*

Conditions	D3S1358	VWA	D16S539	D2S1338	XY	D8S1179	D21S11	D18S51	D19S433	THO1	FGA
4°C, closed container	35*	35	28	28	35	35	35	28	35	35	28
	49†	49	42	42	49	49	49	42	49	49	42
21°C, closed container	28	28	28	28	28	28	28	21	28	28	28
	42	42	35	35	42	42	35	35	42	35	35
21°C, open container	28	21	21	21	28	21	21	21	28	21	21
	35	35	28	28	35	35	35	28	35	35	28
21°C, sand in closed container	35	35	35	35	42	35	35	28	42	35	35
	49	49	42	42	49	49	49	42	49	49	42
21°C, peat soil in closed container	21	14	14	14	21	14	14	7	21	14	14
	28	28	21	21	28	28	28	21	28	28	21
21°C, pond water in closed container	21	21	21	21	28	21	21	14	28	21	21
	35	35	28	28	35	35	35	28	35	35	28
21°C, salt water in closed container	28	28	28	21	35	28	28	21	28	28	21
	42	42	35	42	42	42	35	35	49	42	42

*The value indicate time limits, in which full AmpFlSTR SGM Plus profiles were typed.
†The value indicate time limits, after which no AmpFlSTR SGM Plus profiles were typed.

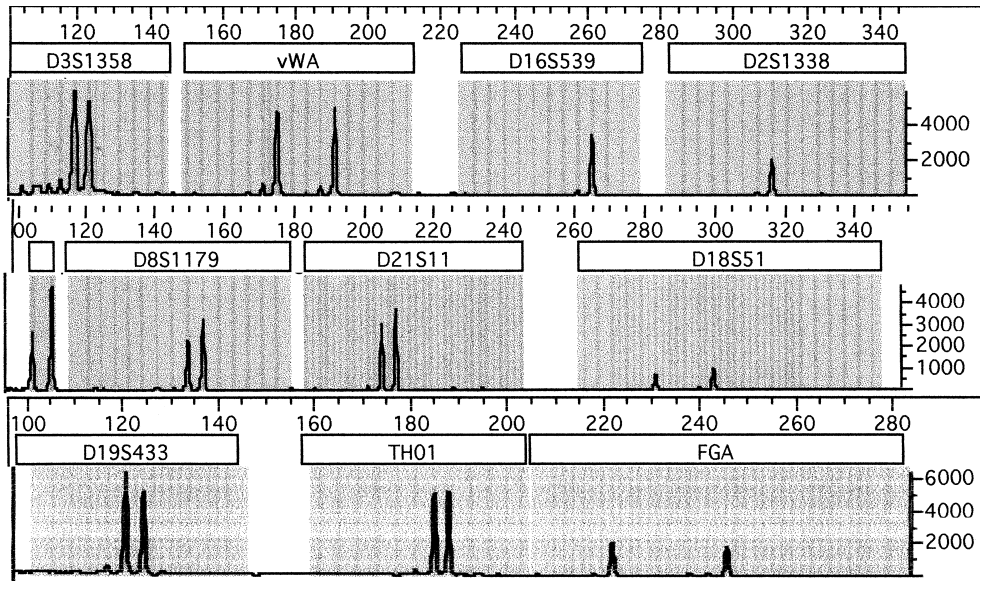

FIG. 1—*Full AmpFlSTR SGM Plus profile obtained from brain tissue specimen incubated for 126 days at 4°C.*

conditions, a corpse may undergo decomposition or preservation (6,7). AmpFlSTR SGM Plus typeability limits for the tissues under study are presented in Tables 1 and 2. Upper values denote time limits in days, when full AmpFlSTR SGM Plus profiles were typeable in all samples. Lower 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 the time spans between the two values, partial profiles were observed. After 14 days of the

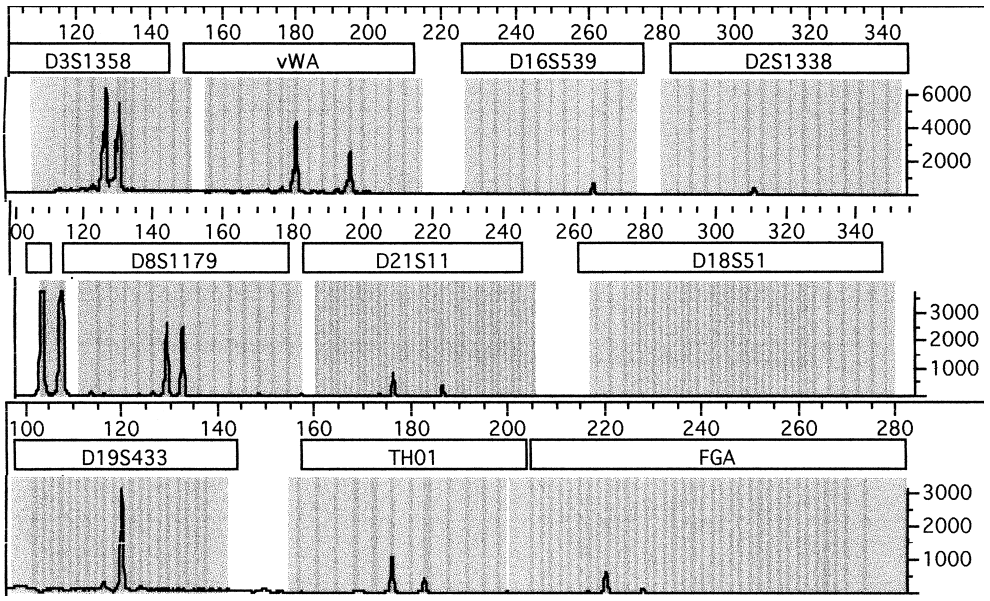

FIG. 2—*Partial AmpFlSTR SGM Plus profile obtained from thyroid gland specimen incubated for 35 days at 4°C.*

experiment, brain tissue specimens incubated at 4°C in closed containers and at 21°C in open and closed containers revealed signs of drying. In the other conditions, on the day 28, thyroid and brain tissue were decomposed. After 56 days, a thick suspension with small tissue fragments were found in water-filled containers. An example of full AmpFlSTR SGM Plus profile is presented in Fig. 1. The Partial profile obtained from a thyroid gland tissue specimen is presented in Fig 2. Fast DNA degradation was observed in the studied material stored in peat soil or sand, which may result from humus acid content, microbial action, or acid pH (8–10). Incubation of brain tissue at 4°C and 21°C in open and closed containers resulted in similar typeability patterns, which may be due to dehydration of the outermost layers of the tissue fragments. Bär et al. (11) described brain DNA as being relatively stable over a period of 3 weeks in comparison with other organs. Ludes et al. (12) was able to confirm this finding for experimental period of 86 days. The investigated model employed in our study does not reflect a typical process of decomposition, as organs extracted from a corpse and placed into a water environment within a short time after death are devoid of body bacteria, contain diluted enzyme activity, and are prevented from air access, which decelerate decomposition process in relation to those in an intact body. In our experiments thyroid gland specimens were less stable for AmpFlSTR SGM Plus typing when compared with brain specimens. This discrepancy may result from a cause of death and/or certain protective mechanisms (1,13).

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