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Effect of soil and water environment on typeability of PowerPlex Y (Promega) in selected tissue samples

Anna Niemcunowicz-Janica[1], Witold Pepinski[1], Jacek Robert Janica[2], Malgorzata Skawronska[1], Jerzy Janica[1], Ewa Koc-Zorawska[1], Ireneusz Soltyszewski[3]

[1]Department of Forensic Medicine, Medical University of Bialystok, Poland
[2]Department of Radiology, Medical University of Bialystok, Poland
[3]Department of Criminalistics and Forensic Medicine, University of Warmia and Mazury, Olsztyn, Poland

Abstract: In cases of decomposed bodies Y chromosomal STR markers may be useful in identification of a male relative. The authors assessed typeability PowerPlex Y (Promega) loci in tissue material stored in water and soil environment. Tissue material was collected during autopsies of five persons aged 20-30 years with time of death determined within the limit of 14 hours. Heart muscle, liver and lung specimens were stored in pond water, sea water, sand and peat soil. DNA was extracted by organic method from tissue samples collected in 7-day intervals. Liver specimens were typeable in all PowerPlex Y loci within 100 days of storage in pond water with gradual decline at DYS392 in sea water. Heart muscle specimens stored in pond water exhibited allelic loss at DYS19, DYS385, DYS389II and DYS392, while all loci were typeable in sea water stored samples. For lung specimens allelic loss was noted throughout the profile. Storage of liver specimens in peat soil for more than 14 days resulted in allelic drop-out, and after 21 days no profiles were typeable. Heart muscle specimens were typeable in all PowerPlex Y systems after 35-day storage in sand, while allelic drop-out and subsequent lack of profiles were noted after 14 and 35 days respectively. Lung specimens stored in garden soil exhibited allelic drop-out and subsequent lack of profiles after 7 and 21 days, respectively. All PowerPlex Y loci were typeable in the latter material in sand up to day 35 with gradual decline of longer amplicons (DYS19, DYS385, DYS389II and DYS392)

Key words: Forensic science - Tissue decomposition - Environmental conditions - DNA typing - PowerPlex Y

Introduction

In homicide cases, it is not uncommon to reveal victim's body concealed for several months in the ground of a secluded place. 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 including age and weight, antemortem diseases and injuries [1]. Genetic identification of human corpses and remains submitted to decomposition is usually based on DNA samples extracted from the most resistant tissues (hair, bones and teeth) [2,3]. In our previous research typeability of AmpFlSTR SGM Plus loci from soft tissue from selected tissue samples incubated in water and soil environment was investigated [4,5]. The aim of this study was assessment of typeability of STR loci included in PowerPlex Y System in heart, lungs and liver specimens depending on different environmental conditions.

Correspondence: A. Niemcunowicz-Janica, Dept. of Forensic Medicine, Medical University of Bialystok, Waszyngtona Str. 13, 15-230 Bialystok, Poland; tel.: (+4885) 7485950, fax.: (+4885) 7485985, e-mail: annaniem@poczta.onet.pl

Materials and methods

Heart, lungs, and liver specimens were collected during autopsies of five persons aged 20-30 years with post mortem interval (PMI) limited to 14 hours according to recommended anatomical body sections (thorax and abdomen). All the persons died due to hypothermia and early signs of body decomposition were prevented by storage in morgue refrigerator. Tissue specimens of dimensions 2×2×2cm 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 hrs in 0.5 ml digest buffer pH=7.5 (10 mM Tris-HCl, 10mM EDTA, 50 mM NaCl, 2% SDS)

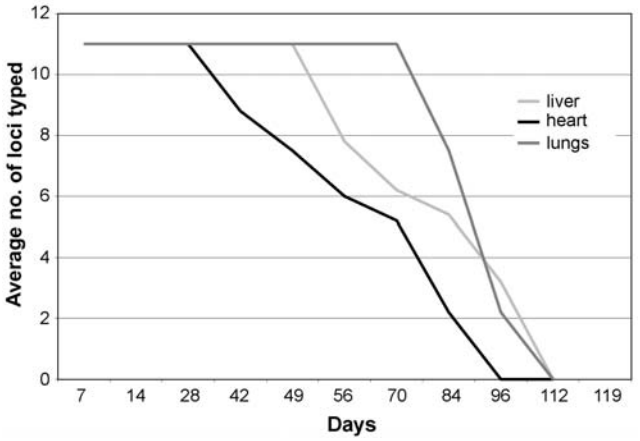

Fig. 1. Average numbers of successfully typed PowerPlex Y loci in tissue specimens incubated in pond water.

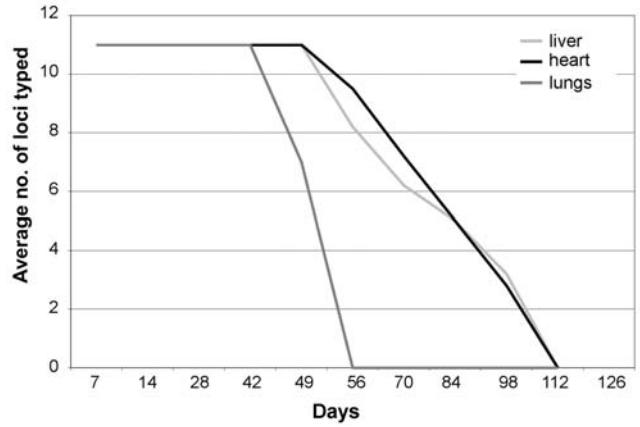

Fig. 2. Average numbers of successfully typed PowerPlex Y loci in tissue specimens incubated in salt water.

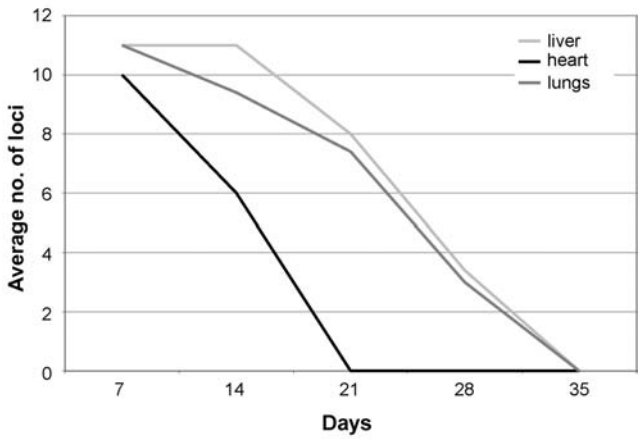

Fig. 3. Average numbers of successfully typed PowerPlex Y loci in tissue specimens incubated in peat soil.

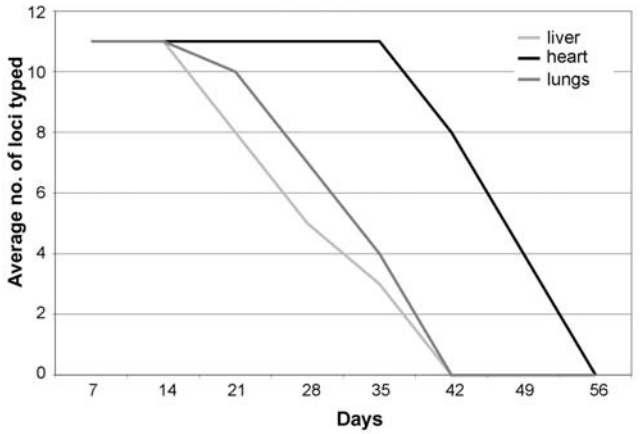

Fig. 4. Average numbers of successfully typed PowerPlex Y loci in tissue specimens incubated in sand.

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 [6,7]. DNA quality was assessed by ethidium bromide 2% agarose gel electrophoresis. Twelve polymorphic Y-STR systems: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439 included in PowerPlex Y-system were amplified following the manufacturer's instructions (Promega) 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, USA) using Genescan v3.11 and Genotyper v2.5 software. As a threshold value a signal of 150 RFU was assumed. Average numbers of successfully typed respective PowerPlex Y-STR loci were assessed for each of five samples of decomposing tissue specimens in consecutive time intervals.

Results

Extracted DNA yield ranged 0-5 ng. Full PowerPlex Y haplotypes were typeable in the specimens immersed in salt water within 56 days. After 35 days a thick suspension with small heart muscle fragments were found. Subsequent loss of alleles at DYS385, DYS392, DYS389, DYS19, DYS439 and DYS390 was seen after 77 days. No profiles were obtained after 119 days. Incubation of the specimens in pond water resulted in faster DNA degradation than that in salt water (Fig.1, Fig.2). Thus, after 49 days a drop-out of larger allele lengths (DYS385, DYS392, DYS389 and DYS19) was observed and after 98 days only two specimens could be typed for DYS391 and DYS393. After 21 days, a thick suspension with small lung fragments were found. The specimens immersed in pond water and in salt water were typeable in all PowerPlex Y Plus loci within 84 and 49 days, respectively. After 112 days only DYS391 and DYS438 were typed in the specimens immersed in pond water. No allele peaks

were seen after 126 days. After 56 days of the experiment profiles from lung specimens immersed in salt water exhibited a gradual allelic loss DYS385, DYS392, DYS389 and DYS19 with no allele peaks seen after 70 days. Liver specimens were typeable in all PowerPlex Y loci in pond water and in salt water up to days 42 and 56, respectively. Gradual decline of longer amplicons and subsequent lack of haplotypes was noted up to the day 126 in both water environments.

Heart muscle specimens incubated in peat soil underwent fast decomposition and became liquefied. The process was also reflected by faster DNA degradation. After 14 days no largest allele peaks (DYS385, DYS392 and DYS389II) could be typed and after 21 days no peak signals were observed. Full PowerPlex Y haplotypes were typeable within 35 days in heart muscle specimens stored in sand. Gradual decline of longer amplicons was noted up to day 56. Incubation of liver and lung specimens in peat soil resulted in similar typeability patterns, respectively, however DNA degradation rate in sand was slower when compared to that in peat soil. Full haplotypes were typeable from all the samples collected within 14 days of incubation with subsequent loss of DYS385, DYS392, DYS389II, DYS19, DYS439, DYS390 and DYS437 peaks. After 28 days only DYS391, DYS438 and DYS393 were typeable in single specimens. No alleles were observed after 35 days. Up to day 28 of the experiment the haplotypes gradually lacked DYS385, DYS392, DYS389II and DYS19 alleles. After 28 days only DYS391, DYS438, DYS393 and DYS389I were typeable in single specimens. No allele peaks were seen in the liver and lung specimens after 42 days (Fig. 3 and 4).

Discussion

The authors evaluated typeability of PowerPlex Y-STR loci in tissue heart muscle, lung and liver specimens incubated at 21°C in water and soil environmental conditions. DNA was extracted using the organic method, commonly employed in genetic identification of mass disaster victims [8,9]. Soil conditions are reported to decelerate postmortem processes, on the other hand its organic substances, humus acids in particular, may inhibit enzymatic DNA amplification [10]. Attempts to remove these inhibitors have been reported [10-12]. On the other hand the organs extracted from a corpse and placed into a water environment within a short time after death is devoid of body bacteria, contains diluted enzyme activity and is prevented from air access, which decelerates decomposition process in relation to that in an intact body. The presented results correspond with those obtained by genotyping AmpFlSTR SGM Plus loci kit in terms of typeability of similar fragment lengths [4,5].

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