TECHNICAL NOTE

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A Standard of AmpliType PM Typing from Aged Evidentiary Samples

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ABSTRACT: In analyzing aged samples by the AmpliType PM PCR amplification and Typing kit, it was occasionally observed that color developed typing strips had dark allele dots on PM loci but no visible S dot. Since the S dot acts as a minimum dot intensity control to determine positive alleles on the PM loci, it is necessary to apply another control system. To achieve positive PM typing from a degraded DNA sample that is inferred to be derived from a single donor, a standard has been adopted wherein loci from which sufficient PCR products are observed on agarose gel can be typed. The objective determination of sufficient PCR was done by comparison between band peak height of each locus generated from a sample and that of the corresponding locus generated from two nanograms (recommended minimum quantity as template DNA) of the control DNA provided in the kit.

KEYWORDS: forensic science, PCR, DNA typing, aged samples, degraded DNA, AmpliType PM

The AmpliType PM PCR amplification and typing kit (the PM kit) developed by Roche Molecular Systems has been commercially available as a forensic DNA typing kit in Japan since 1994. Acceptability of this kit for casework evidence has been evaluated by the forensic science community (1–3). However, a strategy of interpreting strips that have visible dots on PM loci without the visible S dot has not been reported. Since aged samples that contain degraded DNA occasionally produce strips that have dark dots on PM loci without the visible S dot, it would be necessary to apply another control system for each PM locus. The aim of this paper is to present a technical strategy in applying the PM typing kit for analyzing evidentiary samples that contain degraded DNA.

Materials and Methods

Samples

The DNA was purified by phenol-chloroform from 83 bloodstains on gauze, paper, and cotton, and stored in five laboratories in Japan for a period of 15 to 37 years at room temperature. Each sample was derived from a single person. The concentrations of the DNA were determined using a D17Z1 probe (Quantiblot Human DNA Quantitation Kit, Perkin Elmer, Foster City, CA).

PCR Amplification and Typing

The five PM loci (LDLR (4), GYPA (5), HBGG (6), D7S8 (7), GC (8)) and the HLADQA1 (9) locus were amplified and detected by using the AmpliType PM PCR Amplification and Typing Kit (Perkin Elmer, Foster City, CA) in accordance with the manufacturer's protocol. As an exception, when the DNA concentration of the aged sample was less than 0.1 ng/μL, 20 μL (the maximum volume to be added to the PCR mixture) of the extracted samples were used for amplification, resulting in less than 2 ng (the minimum quantity recommended by the protocol) of template DNA.

Verification of PCR Products

The amplified products from the aged bloodstains and control DNA provided in the kit were analyzed on 3% Nusieve GTG + 1% Seakem GTG (FMC, RockLand, ME) agarose gel following the manufacturer's protocol to determine which loci had been amplified. After the minigel had been run, DNA separated on the gel was photographed under UV illumination. To provide a positive comparison of DNA quantities amplified from the aged samples and control DNA, peak heights of DNA bands on the negative films were measured with a laser-based densitometer (Personal Densitometer SI Image QuaNT, Molecular Dynamics, Sunnyvale, CA). Furthermore, in order to determine the band peak height variations between PCR for control DNA solutions, PCR was performed in duplicate with two control DNA solutions from separate PM kits having the same lot number. These four samples were analyzed using agarose gel electrophoresis in quadruplicate.

Results and Discussion

The S dot is designed to produce minimum dot intensity to act as a control for the PM loci to determine which alleles are present. Figure 1 shows developed PM DNA Probe Strips obtained from 22-year-old bloodstains. While none of those strips had visible S dots, dark dots appeared on the HBGG, D7S8, and GC loci. In contrast, light or faint dots appeared on the LDLR and GYPA loci. Intensities of two allele dots within a locus were well-balanced and

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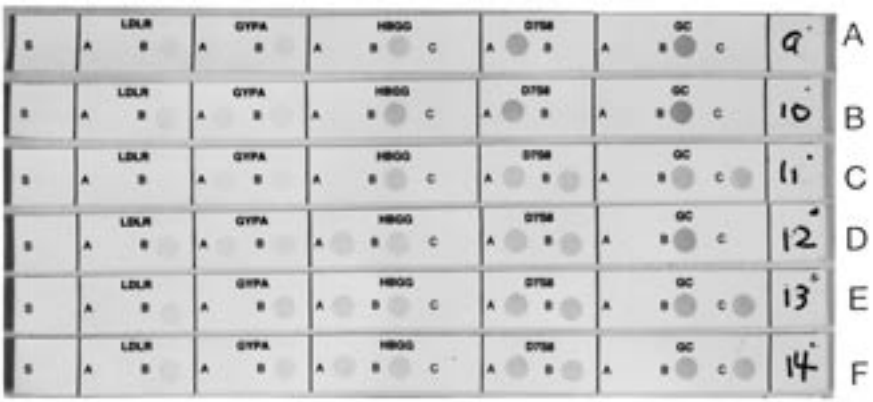

FIG. 1—*PM typing of PCR products amplified from 22-year-old bloodstains: A to F: Subjects; PCR products produced dark dots on HBGG, D7S8, and GC loci without the visible S dots on strips.*

TABLE 1—*The comparison of detectability from aged samples among 6 loci.*

		No. of Samples						
			A Dark Dot/Dots Detected at					
Age of Samples (year)	No. of Tested Samples	The S Dot Detected DQA1* (239/242 bp)†	LDLR (214 bp)†	GYPA (190 bp)†	HBGG (172 bp)†	D7S8 (151 bp)†	GC (138 bp)†	No Dot Detected
17–20	15	12	12	12	12	15	15	0
21–25	48	5	12	12	27	30	35	13
26–29	15	2	3	3	7	8	8	7
33 & 37	5	0	0	0	0	0	0	5

* The probe on the S dot is identical in DNA sequence to that of the C dot on AmpliType HLADQα strip; † The size of the PCR product.

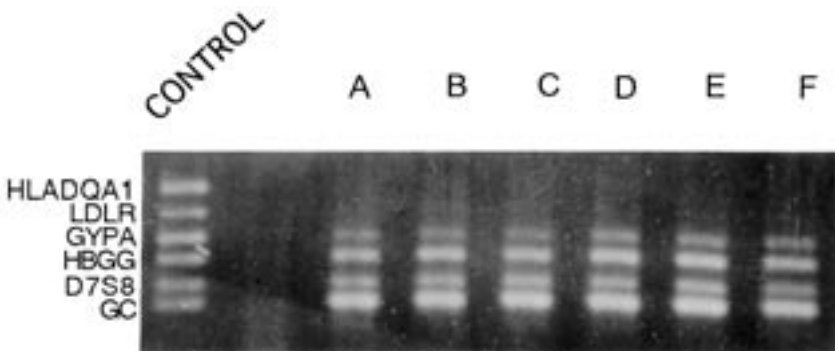

FIG. 2—*Verification of PCR amplification from 22-year-old bloodstains by agarose gel: A to F: The same subjects as in Fig. 1; Control: 2 ng of control DNA provided in PM kit; The bands for GYPA, HBGG, D7S8, and GC were observed on agarose gel utilizing PCR product amplified from 22-year-old bloodstains.*

not more than two alleles were observed per locus per sample, being consistent with the fact that each of them was derived from a single person. Table 1 shows the result of PM strips obtained from the 83 aged bloodstain samples. While 19 out of the 83 samples produced dark dots on all PM loci with the S dot, 25 samples produced no visible dots at all. The remaining 39 samples produced no visible S dots, however, they produced dark dots on PM loci on the strips.

The sequence of the probe immobilized on the S dot is complementary to none of the PCR products of the PM loci. It is, however, complementary to that of HLADQA1. In general, small target DNA sizes increase the success rate of obtaining PCR product from degraded DNA samples (10,11). Since PCR product sizes of PM loci (ranging from 138 to 214 bp) are smaller than that of HLADQA1 (239/242 bp), it is possible that the same DNA sample is sufficient to amplify PM loci but too degraded to amplify the HLADQA1 locus. The degree of DNA degradation in the 39 samples that produced strips being uninterpretable due to an absence of the visible S dot was inferred at that level. Figure 2 shows the result of agarose gel electrophoresis verifying PCR amplification from 22-year-old bloodstains derived from subjects A to F, which were the same samples as in Fig. 1. The bands corresponding to the loci which produced a dark dot or dots appeared equal to or more intense than those generated from 2 ng of the control DNA. It was

recognized that sufficient amounts of PCR products were present in postamplified specimens to perform PM typing of the relevant loci. Hence, this observation enabled us to predict a complete genotype as a result. Therefore, when PCR products from an evidentiary sample are compared to PCR products from a known positive control, it is possible to predict successful typing of the evidentiary sample. Thus, the standard has been adopted of positively typing the loci for which sufficient PCR products are observed on agarose gel, and sufficiency being determined based on amounts generated from the control DNA.

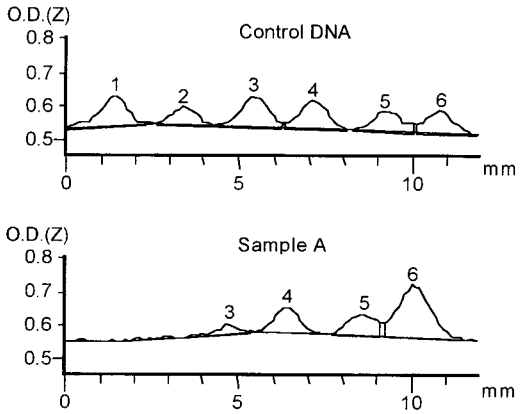

FIG. 3—*Line graph providing a visual display of the pixel intensities determined by the quantitation: Using the software, peaks were recognized while the base line was established. Drops lines indicate the boundaries between peaks. Top: Control DNA; Bottom: Sample A; Peaks 1, 2, 3, 4, 5, and 6 correspond to HLADQA1, LDLR, GYPA, HBGG, D7S8, and GC, respectively.*

TABLE 2—*Coefficient of variation of band peak height on each locus within a gel.*

	Electrophoresis (gel)			
Locus	1	2	3	4
DQA1	14.1	13.4	6.4	10.9
LDLR	17.6	11.0	15.6	11.1
GYPA	14.1	13.1	10.2	1.9
HBGG	15.1	13.4	8.1	5.2
D7S8	3.3	8.2	8.4	7.2
GC	9.6	9.0	8.5	8.1

NOTE: Coefficient of variation of band peak height on each locus within a gel is calculated using four post-amplified specimens obtained from two control DNA solutions.

To compare all bands of DNA samples with relevant bands of Control DNA for intensity, band peak heights of DNA samples and Control DNA were quantitated by instrumentation. Figure 3 shows Peak height quantitation by laser-based densitometer. Since it was apparent that variability of band peak heights between PCR for the control DNA solutions existed, coefficient of variation (CV) of band peak height on each locus was calculated from each of four independent agarose gel electrophoreses using four amplified specimens obtained from two control DNA solutions (Table 2). The CV values of all loci were approximately 10%. Therefore, we raised the threshold for determination of the loci to type, to 110% of the band peak height of each locus generated from the control DNA, analyzed with aged samples on gel. Table 3 shows values of peak heights generated from the control DNA and samples A to F, as well as threshold values for positive typing. According to the new standard, three loci (HBGG, D7S8, and GC) became typable with samples B, D, and E, two loci (D7S8 and GC) became typable with samples A and C, and the GC locus became typable with sample F. Thus it indicated the usefulness of the new standard. As reported by Budowle et al., there is a limitation for detecting sample mixtures with the PM test [1]. Therefore, the new standard should not be applied to samples which are potentially derived from more than one person. As long as this criterion is observed and PCR and typing procedures are performed in accordance with the manufacturer's protocol, the PM typing results obtained by applying this standard will not lose forensic reliability.

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TABLE 3—*Band peak heights of PM loci on agarose gel measured with a laser-based densitometry.*

	DQA1	LDLR	GYPA	HBGG	D7S8	GC
Control DNA	0.089	0.058	0.094	0.082	0.060	0.062
Threshold	0.098	0.064	0.104	0.091	0.066	0.069
A	…	…	0.037	0.081	0.070*	0.157*
B	…	…	0.040	0.104*	0.069*	0.162*
C	…	…	0.028	0.084	0.075*	0.169*
D	…	…	0.055	0.122*	0.073*	0.172*
E	…	…	0.059	0.129*	0.097*	0.198*
F	…	…	0.036	0.090	0.065	0.148*

NOTE: Threshold is 110% of the bank peak height generated from 2 ng of the control DNA A to F: The same subjects as in Fig. 1; …: No peak recognized; *: the sample was called at the locus by applying the standard.

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