TECHNICAL NOTE

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Validation Studies for the Genetic Typing of the D1S80 Locus for Implementation into Forensic Casework

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ABSTRACT: A series of validation experiments were designed to evaluate, according to the Technical Working Group on DNA Analysis Methods (TWGDAM) guidelines, the analysis of the D1S80 locus for casework implementation. Approximately 400 samples from three different populations (Minnesota Caucasians, Minnesota African Americans, and Minnesota Native Americans) were typed to determine allele frequencies. Simulated forensic type specimens (blood, saliva, hair and semen, or vaginal secretions) were typed to demonstrate that deoxyribonucleic acid (DNA) extracted from various tissues of an individual yield the same D1S80 type.

Dilution studies were performed and it was determined that a wide range of input DNA (0.5 ng to 40.0 ng) will consistently yield typeable results. The evaluation of DNA from various animals showed that the D1S80 locus is specific to human DNA within the limits of the parameters tested. The reproducibility of the system was tested by duplicate analysis of approximately 200 population samples. Duplicate samples were analyzed on both horizontal and vertical gel systems. In addition, simulated forensic specimens were analyzed by two independent laboratories: the Minnesota Forensic Science Laboratory (MFSL) and the Roche Biomedical Laboratories (RBL). All analyses, including extraction, quantitation, amplification and typing, were performed independently. All typing results for both laboratories were in agreement.

By the analysis of mixtures from various simulated casework type mixtures, it was demonstrated that the D1S80 typing system is suitable for analyzing mixtures. In addition to the simulated casework, evidentiary samples from several adjudicated cases previously analyzed by restriction fragment length polymorphism (RFLP) analysis and/or DQA1 were typed at the D1S80 locus. The D1S80 results were consistent with previous RFLP and/or DQA1 results regarding inclusions/exclusions.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, D1S80, validation, variable number of tandem repeats.

Since its discovery, the polymerase chain reaction (PCR) has become a very powerful technology in the analysis of forensic samples (1). Two systems, the commercially available Amplitype HLA DQA1 and Amplitype PM PCR Amplification and Typing kits (Perkin-Elmer, Foster City, CA) have been extensively validated (2–6) and are currently used in forensic laboratories worldwide (7–13). These two systems use sequence differences between the alleles, with a reverse dot blot format (14,15) for the genotyping of DNA samples. Other polymorphic regions of the DNA molecule are typed utilizing length differences of the amplified products (Amplified Fragment Length Polymorphisms—AMP-Flps) (16). The PCR products of AMP-Flps are generally separated using a polyacrylamide gel (16) and the fragments are compared to an allelic ladder or an internal sizing standard for genotyping (30). In 1990, Kasai et al. published the primer sequences for the amplification of the D1S80 locus and also initial population data (17). Many population databases have since been assembled (18–28) and numerous validation studies published (29–33) for the D1S80 locus that confirm that amplification of the D1S80 locus performs as expected under various conditions encountered in forensic casework. The following areas of validation, which demonstrate compliance with aspects of the Technical Working Group on DNA Analysis Methods (TWGDAM) guidelines for quality assurance (34), were addressed by the Minnesota Forensic Science Laboratory (MFSL) in collaboration with the Roche Biomedical Laboratories (RBL), before implementation of the D1S80 system into casework: Population studies, reproducibility studies, standard specimens, minimum sample, nonhuman studies, mixed specimens, and nonprobative cases.

Materials and Methods

Extraction and Quantitation

The DNA from all samples analyzed in these studies were organically extracted as previously described (35), unless otherwise noted. The DNA was quantitated by slot blot hybridization with the primate specific probe p17H8 (36–38) using commercially available kits (Photoprobe Human DNA quantitation system, Gibco BRL, Baltimore, MD or Quantiblot Human DNA Quantitation Kit, Perkin-Elmer, Branchburg, NJ) and following the recommended procedures supplied with the kits.

Amplification

Three different protocols for the amplification of the D1S80 locus were used by the two laboratories and are outlined below:

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MFSL PROTOCOL 1—This protocol was used to amplify population samples and mixed specimen study samples.

Primer sequences (purchased from Operon Technologies, Inc., Alameda CA):

pMCT118-1: GAAACTGGCCTCCAAACACTGCCCGCCG
pMCT118-2: GTCTTGTTGGAGATGCACGTGCCCCTTGC

Reaction mixture components in 50 μL volume:

5–10 ng DNA
1X PCR Buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.001% gelatin)
1.5 mM $MgCl_2$
200 μM of each of the four deoxyribonucleoside triphosphates
0.2 μM of each primer
1.0 U of Taq DNA polymerase

Amplification procedure using a Geneamp 9600:

Segment 1: 95°C for 10 s
Segment 2: 67°C for 10 s
Segment 3: 70°C for 60 s
Program for 27 cycles

MFSL PROTOCOL 2—This protocol was the same as MFSL Protocol 1, except that it was done in a 20 μL reaction volume instead of 50 μL. This protocol was used to amplify population samples, standard biological specimens, dilution samples, nonhuman samples, and nonprobative casework samples.

RBL PROTOCOL—This protocol was used to amplify mixed specimen samples.

Primer sequences (synthesized in-house):
pMCT118-1: AAACTGGCCTCCAAACACTGCCCGCCG
pMCT118-2: TCTTGTTGGAGATGCACGTGCCCCTTGC

Reaction mixture components in 100-μL volume:

5–10 ng DNA
1X PCR Buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.001% gelatin)
1.5 mM $MgCl_2$
200 μM of each of the four deoxyribonucleoside triphosphates
0.2 μM of each primer
2.5 U of Taq DNA polymerase

Amplification procedure using a Geneamp 9600:

Hot Start: 94°C for 2 min 30 s
Segment 1: 94°C for 30 s
Segment 2: 65°C for 30 s
Segment 3: 72°C for 2 min
Program for 25 cycles

Final extension: 72°C for 3 min

Slow cool: Go to 50°C approximately 1°C per 30 s. Hold at 50°C for 30 s. Go to 15°C approximately 1°C per s. Hold at 15°C.

Horizontal Electrophoresis (MFSL)

Ultra-thin layer polyacrylamide gels (0.4 mm) were cast onto GelBond® PAG (FMC Bioproducts, Rockland, MD) using the "flap technique (39)." The 12 by 20 cm acrylamide gel [7.5% acrylamide, 2% piperazine diacrylamide, 60 mM formate and 37.5 mM Tris-HCl, pH 9.0, containing 24 μL N,N,N′,N′ tetramethylethylenediamine (TEMED) and 240 μL 10% ammonium persulfate per 24 mL of solution] was allowed to polymerize at least 2 h at room temperature. The gel was placed into an isothermal controlled electrophoresis system (Model EC1001, VWR Scientific, St. Louis, MO). Approximately 5 μL of PCR product was loaded onto fiberglass sample applicator tabs (Pharmacia LKB Biotechnology, Piscataway, NJ). Blot pads (Gibco BRL) soaked in 2X Tris-borate buffer [0.28 M borate, 1.04 M Tris-HCL, (pH 9.0) and 0.00005% bromophenol blue] provided the trailing ion. The blot pads were placed 20 cm apart and electrophoresis was performed until the tracking dye had traveled 19–20 cm (approximately 2.5 h). Control limits for the electrical settings were 600 V, 25 mA, and 20 W. Gels were then silver stained using the procedure previously described (16).

Vertical Electrophoresis (MFSL)

Polyacrylamide gels (0.4 mm) were poured between two glass plates using modifications of the gel pouring techniques previously described (39–42). GelBond® was affixed to the long glass plate (19.5 by 36 cm) with the hydrophobic side down. Spacers (0.4 mm) were attached to the GelBond® with a thin layer of high vacuum grease (Dow Corning Corp, Midland, MI). The acrylamide mixture [7.5% acrylamide, 2% piperazine diacrylamide, 60 mM formate and 37.5 mM Tris-HCl, pH 9.0, containing 32 μL N,N,N′,N′ tetramethylethylenediamine (TEMED) and 320 μL 10% ammonium persulfate per 32 mL of solution] was pipetted next to the spacer for the length of the gel. The short glass plate (19.5 by 36 cm) was then slowly "flapped" down to spread the acrylamide between the plates. A 20-tooth delrin comb (0.4 mm thick, custom made by Gibco BRL) was inserted and held in place by a clamp. The gel was allowed to polymerize for at least 1 h at room temperature. Gel dimensions were 17 by 33.5 cm. The gel was placed into a Model SA-32 sequencing apparatus (Gibco BRL) with 0.5X TBE as the running buffer. The PCR product was combined with loading buffer (40% sucrose, 2.5% bromophenol blue and 2.5% xylene cyanol) (43) in a ratio of 4:1, and 3 μL loaded onto the gel. Control limits for the electrical settings were 1000 V, 150 mA, and 50 W. Electrophoresis was performed until the xylene cyanol tracking dye had traveled 22–25 cm (approximately 1.5–2 h). Gels were then silver stained using the procedure previously described (16).

Vertical Electrophoresis (RBL)—Precast 8% polyacrylamide tris-sulfate gels (0.8 by 17 by 33.5 cm) were purchased commercially (Gibco BRL). The gel was placed into a Model SA-32 sequencing apparatus (Gibco BRL) with 1X TBE as the running buffer. Approximately 15-μL of PCR product was combined with 5 μL loading buffer (40% sucrose, 2.5% bromophenol blue, and 2.5% xylene cyanol) (43) and 15 μL loaded onto the gel. Control limits for the electrical settings were 500 V, 150 mA, and 50 W. Electrophoresis was performed until the xylene cyanol tracking dye had traveled 22–25 cm (approximately 5 h). Gels were then silver stained using the procedure previously described (16).

Typing—Alleles were typed using side by side comparison to an allelic ladder either purchased from Perkin-Elmer or amplified in-house as previously described by Baechtel et al. (30). A K562 control was run on every gel. Anodal (A) and cathodal (C) variants were "binned" with the nominal alleles.

Population Studies—DNA was previously extracted by the method of Dykes et al. (44) from approximately 400 unrelated

Native American, African American, and Caucasian blood samples obtained from the Minneapolis Memorial Blood Center. Allele frequencies were determined from the genotypes observed and compared to previously published data. The data were analyzed for Hardy-Weinberg Equilibrium using the program 'Genepop' described by Raymond and Rousset (45).

Biological Specimens—Biological samples (blood, saliva, hair, and either semen, or vaginal secretions) were collected from eight volunteers. Blood was collected in ethylene diaminetetraacetic acid (EDTA) tubes, spotted onto Schleicher & Schuell 903 paper (S& S 903; Schleicher & Schuell, Keene, NH), allowed to air dry and stored at 4°C. Volunteers "chewed" on sterile gauze until the gauze was saturated. The gauze was allowed to air dry and then stored at 4°C. Aliquots of fresh semen were spotted on S&S 903 paper, allowed to air dry and stored at 4°C. Semen-free vaginal swabs were collected, allowed to air dry, and stored at 4°C. Hair was pulled from the heads of the volunteers. DNA was extracted from the hair root following the organic method outlined in the Amplitype Users Guide (46).

Minimum Sample—DNA was organically extracted from bloodstains of two individuals of known D1S80 types (24,28 and 17,41+). Based on slot blot analysis, appropriate dilutions were made and the following quantities of DNA were amplified as described above: 20.0, 10.0, 5.0, 2.5, 1.25, 0.31, and 0.16 ng.

Nonhuman Studies—Bloodstains from 26 animals, listed in Table 1, were obtained from the University of Minnesota Veterinary School. For each bloodstain, DNA from a 1 cm^2 cutting was extracted. Based on yield gel analysis, approximately 5 ng of DNA from each animal was amplified. An evaluation of the PCR product was performed by analyzing 10 μL of each sample on both agarose and acrylamide gels. A 10 by 16 cm, 2% agarose gel was prepared with 1X Tris-Acetate-EDTA (TAE) (0.04 M Tris-Acetate, 0.001M EDTA) containing ethidium bromide (0.1 μL/mL). Size markers made up of DNA fragments in the size range of 100 to 1500 bp (Gibco BRL) were loaded into the outer wells of each gel. Electrophoresis proceeded at 200 V for 30 min in 1X TAE buffer. The PCR products were examined under ultraviolet light. The acrylamide gels were run as previously described in the Vertical Electrophoresis (MFSL) section.

Reproducibility—Many validations were completed using the horizontal gel electrophoresis system (16); however, during the course of the studies, TWGDAM consensus recommended that D1S80 be analyzed on a vertical gel electrophoresis system (Personal Communication, Terry L. Laber, TWGDAM member). The goal of this reproducibility study was to demonstrate that the same results would be obtained using the vertical system as were obtained using the horizontal system so that those validations completed using a horizontal system would not have to be repeated using the vertical system. Approximately 200 of the population samples were typed using both the vertical and horizontal systems.

TABLE 1—*List of animals for which the DNA obtained was amplified at the D1S80 locus.*

Blue Wing Teal	Horse	Pig
Cat	Lamb	Rabbit
Chevrotain	Llama	Rat
Cow	Mallard	Sheep
Deer	Monkey	Tapir
Dog	Moose	Tiger
Elk	Mountain Goat	Wolf
Goat	Mule Deer	Zebra
Greater Kudu	Musk Ox	

Mixed Specimen Studies—Nine mock sexual assault cases were assembled in duplicate. Six of the cases (Case #1–6) were prepared having a female victim and a male suspect. Two of the cases (Case #7–8) were prepared having a male victim, male suspect and a male elimination sample. The final case (Case #9) was prepared using semen from vasectomized individuals. A summary of the controls and samples analyzed in the mock cases is listed in Table 2. One set of cases was analyzed by the MFSL and the second set of cases was analyzed by RBL. The differential extraction procedure used by the MFSL was previously described by Gross et al. (6) for the extraction of the vaginal swabs. Samples were either diluted or concentrated with a Centricon microconcentrator so that 10 ng of DNA would be in a volume of 10 μL or less.

Non-Probative Casework—Non-probative evidentiary stain material was typed and compared to typing results obtained from submitted known blood samples. The D1S80 results were then compared to RFLP and/or DQA1 results. A summary of the samples analyzed is as follows: Case #1 Submitted known blood sample of suspect compared to blood on glass. Case #2 Submitted known blood sample of suspect compared to blood on gauze removed from glass. Case #3 Submitted known blood sample of victim compared to blood under the left and right hand fingernails of victim.

Results and Discussion

Population Studies—Allele frequencies for three racial databases were calculated from the observed genotypes and are shown in Table 3. Twenty-one different alleles were observed for the Caucasian population. The two most common alleles were the 18 and 24, having allele frequencies of 0.248 and 0.350, respectively. Twenty different alleles were observed for the African American population. The most common alleles were the 18, 21, 24, and 28, having allele frequencies of 0.112, 0.112, 0.196, and 0.143 respectively. Both databases are similar to other published Caucasian and African American databases (26,31) and are significantly different from each other. Eighteen different alleles were observed for the Native American population. The two most common alleles were the 18 and 24, having allele frequencies of 0.329 and 0.322, respectively. Observed and expected number of homozygotes for each racial database are shown in Table 4. The expected numbers of homozygotes or heterozygotes were computed using Levene's correction (47). Based on analyses using 'Genepop,' it was determined that the Caucasian and African American databases were statistically in Hardy-Weinberg Equilibrium. The Native American sample deviated from Hardy-Weinberg expectation. The Native American database is comprised of samples from the Sioux and Chippewa tribes in Minnesota, however, the samples could not be identified as coming from one or the other. This substructuring is the most likely explanation of this database being out of Hardy-Weinberg Equilibrium. However, the expected genotype frequencies are all within the ±95% confidence interval for this database. Because a conservative minimum frequency of five divided by twice the database size (i.e., 5/2N) is used in estimating a genotype

TABLE 2—*Summary of the controls and samples used in the set-up of nine mock forensic cases that were prepared in duplicate and analyzed by the Minnesota Forensic Science Laboratory and by the Roche Biomedical Laboratories.*

Case #	Controls	Swab #1	Swab #2	Swab #3	Swab #4
1	B_1,B_2	V_2	VM_2	$V_2 + S_1$	S_1
2	B_1,B_2	V_2	$VM_2 + S_1$	$O_2 + S_1 + B_2$	S_1
3	B_1,B_2	V_2	$VM_2 + S_1$	$O_2 + S_1$	S_1
4	B_1,B_2	V_2	I	X	S_1
5	B_1,B_2	V_2	I	$O_2 + S_1$	S_1
6	B_1,B_2,B_3	$V_2 + S_1$	$VM_2 + S_1$	$V_3 + S_1$	—
7	B_1,B_2,B_3	A_1	U_2	$A_2 + S_3$	$U_2 + S_3$
8	B_1,B_2,B_3	$A_2 + S_1 + S_3$	$A_2 + S_1 + S_3$	$A_2 + S_1 + S_3$	—
9	B_1,B_2	S^*_2	S^*_2	S^*_1	—

Legend: B = Blood.
S = Semen.
S* = Semen from vasectomized individual.
V = Semen free vaginal swab.
VM = Semen free vaginal swab taken during menstrual cycle.
I = Vaginal swab after vaginal intercourse.
O = Oral swab.
X = Oral swab after oral sex.
A = Semen free anal swab.
U = Urine.

TABLE 3—*D1S80 allele frequencies of the Minnesota racial databases calculated from genotype frequencies. Allele sizes were determined using side by side comparison to an allelic ladder. "N" is the number of samples analyzed.*

Allele	Caucasian $N = 113$	Native American $N = 149$	African American $N = 143$
14	0.0044	0.0000	0.0000
16	0.0000	0.0201	0.0035
17	0.0000	0.0000	0.0245
18	0.2477	0.3289	0.1119
19	0.0044	0.0335	0.0035
20	0.0132	0.0168	0.0315
21	0.0310	0.0168	0.1119
22	0.0663	0.0269	0.0804
23	0.0177	0.0134	0.0105
24	0.3496	0.3222	0.1958
25	0.0310	0.0235	0.0559
26	0.0177	0.0201	0.0000
27	0.0044	0.0067	0.0210
28	0.0354	0.0269	0.1433
29	0.0398	0.0336	0.0664
30	0.0089	0.0201	0.0140
31	0.0663	0.0772	0.0594
32	0.0089	0.0067	0.0105
33	0.0000	0.0000	0.0035
34	0.0177	0.0000	0.0420
36	0.0132	0.0000	0.0035
37	0.0132	0.0000	0.0000
40	0.0044	0.0034	0.0070
41	0.0044	0.0034	0.0000

TABLE 4—*Observed and expected heterzygote and homozygote classes for D1S80 in Minnesota populations.*

	Caucasians	African Americans	Native Americans
Heterozygotes Observed	89	125	111
Heterozygotes Expected	91	128	116
Homozygotes Observed	24	18	38
Homozygotes Expected	22	15	33

frequency for forensic purposes, the Native American database is suitable for deriving frequency estimates for forensic purposes.

Biological Samples—PCR products from the amplication of DNA from the blood, saliva, hair, and either semen or vaginal secretions were run on vertical polyacrylamide gels. The results demonstrate that the DNA extracted from different biological sources of an individual and analyzed at the D1S80 locus give consistent results. The typing of the D1S80 locus therefore meets the TWGDAM guidelines regarding the consistency of DNA isolated from different tissues within the same individual yielding the same type (34).

Minimum Sample—For the sample with a D1S80 type of 24,28, successful amplification and typing were obtained for the 0.31 to 20.0 ng input DNA range. For the sample with a D1S80 type of 17,41+, successful amplification and typing was obtained for the 0.16 to 5.0 ng range. However, "allele dropout" of the 41+ allele occurred when 10.0 ng of DNA was in the amplification reaction and no amplification of either the 17 or 41+ allele was seen when the input amount of DNA was 20.0 ng. However, to obtain a concentration of 20.0 ng of DNA in the reaction mixture, more than 20% of the volume was DNA in 1X TE. Therefore, the reaction may have been inhibited by excess EDTA. This study was repeated on the sample with D1S80 type 17,41+ using the AmpliFlp D1S80 Amplification Kit with the following modifications: 16 μg bovine serum albumin (BSA, Sigma Chemical Company, St. Louis, MO) was added to the reaction and the amount of DNA in 1X TE added was kept below 20% of the volume. For this second study there was successful amplification of both alleles for a DNA range of 0.16 to 40.0 ng. The difference in results between the two studies seemed to be in the addition of BSA to the PCR reaction in the second study. As a result, BSA is routinely added to all sample amplifications and the target amount of input DNA was selected to be 2–5 ng.

Nonhuman Studies—After amplification, all samples were analyzed on both on agarose and a polyacrylamide gel. No bands were visible on either gel type. These results differ from those

published by Baechtel (32); however, the amount of input DNA utilized in each study was significantly different. In the present study, 5 ng of DNA was added to the PCR reaction as opposed to the 124 ng added for most of the samples in the Baechtel study. We chose to put 5 ng DNA into the PCR reaction since that is the approximate amount of DNA (1–10 ng) we put in the reaction for all other samples in this study.

Reproducibility—D1S80 typing results of population samples analyzed on horizontal gels were compared to the results of the population samples analyzed on vertical gels. A considerable number of D1S80 alleles analyzed using horizontal electrophoresis migrated anodically or cathodically to the allelic ladder. To compare those samples analyzed using horizontal electrophoresis to those analyzed using vertical electrophoresis, the off-ladder variants observed using horizontal electrophoresis were binned with the nominal allele. The exception to this was those variants which were midpoint between two nominal alleles, which were termed "M" variants. Other than the "M" variants, all samples typed the same on the horizontal and vertical gel system. Of the 406 alleles analyzed on both gel systems, nine were "M" variants. Of these nine "M" variants, four were 22M, three were 23M, and two were 25M. When the "M" variants were analyzed on vertical gels, the four 22M samples migrated the same distance as the 22 allele in the ladder, and the three 23M samples migrated the same distance as the 23 allele in the ladder; however, the two 25M samples migrated the same distance as the 26 allele in the ladder. This difference in migration of the "M" variants (seven to the lower allele and two to the upper allele) can most likely be accounted for by base pair variation in the repeat sequence (Personal communication, Rebecca Reynolds, Ph.D., Human Identity Section Manager, Roche Molecular Systems, Inc.) It should be noted that all "M" variants were in the Native American population.

Mixed Specimen Studies—Two identical sets of nine mock sexual assault cases were evaluated using D1S80 typing by the MFSL and RBL. A summary of the results follows:

Bloodstains—DNA extracts from eighteen bloodstains were typed by each laboratory at the D1S80 locus. The D1S80 typing results obtained for all corresponding samples were the same.

Semen (neat)—DNA extracts from five semen containing swabs were typed by the MFSL and RBL at the D1S80 locus. Results were compared with the D1S80 results from corresponding known bloodstains. No discrepancies between the D1S80 types obtained from the ten semen swabs (sperm cell fraction) and their corresponding blood samples were observed. D1S80 types were detected in the non-sperm cell fractions for 8 of the 10 swabs with no discrepancies when compared to the D1S80 types obtained from their corresponding bloodstains.

DNA extracts from three swabs containing seminal fluid from vasectomized males were typed by each laboratory, and the results compared with the D1S80 types from corresponding bloodstains. Sufficient DNA for amplification was obtained from the non-sperm fraction for 2 of the 6 swabs. The D1S80 types obtained from the two swabs matched the D1S80 types obtained from their corresponding bloodstains. No DNA was obtained from the sperm cell fraction of any of the swabs containing seminal fluid from vasectomized males.

Vaginal (neat)—DNA extracts from six vaginal swabs were typed by each laboratory and compared with the D1S80 types from corresponding bloodstains. No discrepancies between the D1S80 types obtained from the vaginal secretions and blood were observed for any of the samples.

Mixed stains—DNA extracts from twelve mixed stains were typed and compared with the D1S80 results obtained from corresponding bloodstains of the donors of the components for the mixtures. The mixtures contained various combinations of vaginal secretions, semen, blood, and saliva. The performance of the differential extraction procedures on these twelve mixed stains were evaluated based upon carry-over of non-sperm cell DNA into the sperm cell fraction or carry-over of sperm cell DNA into the non-sperm cell fraction. For the differential extraction performed by the MFSL, 11 of the 12 sperm cell fractions gave D1S80 types that were consistent with the semen donor only and one was a mixture consistent with DNA from the two donors. All 12 of the non-sperm cell fractions gave D1S80 types consistent with a mixture of DNA from the two donors. For the differential extraction performed by the RBL, 6 of the 12 sperm cell fraction gave D1S80 types that were consistent with the semen donor only, and 3 of the 12 were mixtures consistent with DNA from the two donors. For 3 of the 12 there were no results because of insufficient DNA. There was insufficient DNA for these three samples because the DNA was depleted by RFLP analysis, DQA1 typing, PM typing and HUMTH01 typing (48). Since all the "donors" were not available to give samples, additional mock cases could not be prepared for further testing by the RBL. The samples extracted by the MFSL were typed with only PM and D1S80. Five of the 12 non-sperm cell fractions gave D1S80 types consistent with the non-semen donor, and 7 of the 12 were mixtures consistent with DNA from the two donors. For those samples in which there was a mixture in the sperm cell fraction, there was a

TABLE 5—*Comparison of D1S80 typing results and RFLP and/or DQA1 typing results from evidence stains from three non-probative cases.*

Case Samples	RFLP Results	DQA1 Results	D1S80 Results
Item 1A—known blood Item 1B—blood on glass	3 probe (D2S44, D4S139, D10S28) match between 1A & 1B	Item 1A (3,3) matches Item 1B (3,3)	Item 1A (16,22) matches 1B (16,22)
Item 2—known blood Item 3—blood on glass	Insufficient DNA	Item 2 (1.1,1.1) matches Item 3 (1.1,1.1)	Item 2 (24,29) matches Item 3 (24,29)
Item 29—known blood Item 31M—blood under L. fingernails Item 31N—blood under R. fingernails	4 probe (D2S44, D1S7, D14S13, D4S139) match between 29, 31M, and 31N	Item 29 (1.2,3) matches Item 31M (1.2,3) & Item 31N (1.2,3)	Item 29 (18,18) matches Item 31M (18,18) & 31N (18,18)

definite difference in band intensities, so that major and minor components of the mixture could be assigned.

Non-Probative Casework—D1S80 typing results on seven evidence items from three non-probative cases were consistent with the results obtained by other DNA typing methods previously performed on these cases with regard to matches or non-matches. The DNA typing results of these cases are summarized in Table 5. All cases were inclusions for either RFLP and/or DQA1 as well as D1S80.

Conclusion

The studies presented in this paper concur with those previously published (30–32) in that all studies demonstrate that the D1S80 locus is an extremely reliable and robust system and is suitable for use in forensic casework. This is especially evident in this study because correct typing results were obtained on duplicate samples analyzed by independent laboratories on a variety of biological samples. In addition, this study demonstrates that laboratories can employ various protocols for extraction, quantitation, amplification, and gel electrophoresis and still obtain accurate and reproducible results.

References

1. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, et al. Enzymatic amplification of β-Globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985;230:1350–4.
2. Comey CT, Budowle B. Validation studies on the analysis of the HLA DQα locus using the polymerase chain reaction. J Forensic Sci 1991;36:1633–48.
3. Hochmeister MN, Budowle B, Jung J, Borer UV, Comey CT, Dirnhofer R. PCR-based typing of DNA extracted from cigarette butts. Int J Leg Med 1991;104:228–33.
4. Walsh PS, Fildes N, Louis AS, Higuchi R. Report of the blind trial of the Cetus AmpliTaq™ HLA DQα forensic deoxyribonucleic acid (DNA) amplification and typing kit. J Forensic Sci 1991; 36:1551–6.
5. Budowle B, Lindsey JA, DeCou JA, Koons BW, Giusti AM, Comey CT. Validation and population studies of the loci LDLR, GYPA, HBGG, D7S8 and Gc (PM loci) and HLA-DQα using a multiplex amplification and typing procedure. J Forensic Sci 1995; 40:45–54.
6. Gross AM, Guerrieri RA. HLA DQA1 and PM validations: Standard specimens, reproducibility and mixed specimens. J Forensic Sci 1996;41:1022–6.
7. Reynolds R, Sensabaugh G, Blake E. Analysis of genetic markers in forensic DNA samples using the polymerase chain reaction. Anal Chem 1991;63:2–15.
8. Schneider PM, Rittner C. Experience with the PCR-based HLA-DQα DNA typing system in routine forensic casework. Int J Leg Med 1993;105:295–9.
9. Blake E, Mihalovich J, Higuchi R, Walsh PS, Erlich H. Polymerase chain reaction (PCR) amplification and human leukocyte antigen (HLA)-DQα oligonucleotide typing on biological evidence samples: casework experience. J Forensic Sci 1992;37:700–26.
10. Comey CT, Budowle B, Adams EW, Baumstark AL, Lindsey JA, Presley LA. PCR amplification and typing of the HLA DQα gene in forensic samples. J Forensic Sci 1993;38:239–49.
11. Presley LA, Baumstark AL, Dixon A. The effects of specific latent fingerprint and questioned document examinations on the amplification and typing of the HLA DQ alpha gene region in forensic casework. J Forensic Sci 1993;38:1028–36.
12. Herrin G, Jr, Fildes N, Reynolds R. Evaluation of the AmpliType™ PM DNA test system on forensic case samples. J Forensic Sci 1994;39:1247–53.
13. Hochmeister MN, Budowle B, Eisenberg A, Borer UV, Dirnhofer R. Using multiplex PCR amplification and typing kits for the analysis of DNA evidence in a serial killer case. J Forensic Sci 1996;41:155–62.
14. Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA. Analysis of enzymatically amplified B-globin and HLA-DQα DNA with allele-specific oligonucleotide probes. Nature 1986;324:163–6.
15. Saiki RK, Walsh PS, Levenson CH, Erlich HA. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. Proc Natl Acad Sci USA 1989;86:6230–4.
16. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC. Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 1991;48:137–44.
17. Kasai K, Nakamura Y, White R. Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application of forensic science. J Forensic Sci 1990;35:1196–200.
18. Sajantila A, Budowle B, Ström M, Johnsson V, Lukka M, Peltonen L, et al. PCR Amplification of Alleles at the D1S80 Locus: Comparison of a Finnish and a North American Caucasian population sample, and forensic casework evaluation. Am J Hum Genet 1992;50:816–25.
19. Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkman B. Population genetics and forensic data of 4 AMPFLP's. Int J Legal Med 1992;104:329–33.
20. Schnee-Griese J, Bläß G, Herrmann S, Schneider H, Förster R, Bäßler G, et al. Frequency distribution of D1S80 alleles in the German population. Forensic Sci Int 1993;59:131–6.
21. Chuah SY, Tan WF, Yap KH, Tai HE, Chow ST. Analysis of the D1S80 locus by amplified fragment length polymorphism technique in the Chinese, Malays and Indians in Singapore. Forensic Sci Int 1994;68:169–80.
22. Kloosterman AD, Budowle B, Daselaar P. PCR-amplification and detection of the human D1S80 VNTR locus. Int J Leg Med 1993;105:257–64.
23. Guowski S, Budowle B, Auer J, vanOorschot R. Statistical analysis of an Australian population for the loci Gc, HLA-DQA1, D1S80 and HUMTHO1. Forensic Sci Int 1995;76:1–6.
24. Hayes JM, Budowle B, Freund M. Arab population data on the PCR-based loci: HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, and D1S80. J Forensic Sci 1995;40:888–92.
25. Turowska B, Sanak M. D1S80 VNTR locus genotypes in population of South Poland; meta-analysis pointer to genetic disequilibrium of human populations. Forensic Sci Int 1995;75:207–16.
26. Budowle B, Baechtel FS, Smerick JB, Presley KW, Giusti AM, Parsons G, et al. D1S80 population data in African Americans, Caucasians, Southeastern Hispanics, Southwestern Hispanics, and Orientals. J Forensic Sci 1995;40:38–44.
27. Woo KW, Budowle B. Korean population data on the PCR-based loci LDLR, GYPA, HGBB, D7S8, Gc, HLA-DQA1, and D1S80. J Forensic Sci 1995;40:645–8.
28. Scholl S, Budowle B, Radecki K, Salvo M. Navajo, Pueblo, and Sioux population data on the loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, and D1S80. J Forensic Sci 1996;41:47–51.
29. Nakamura Y, Carlson M, Krapcho K, White R. Isolation and mapping of a polymorphic DNA sequence (pMCT118) on chromosome 1p (D1S80). Nucleic Acids Res 1988;16:9364.
30. Baechtel FS, Smerick JB, Presley KW, Budowle B. Multigenerational amplification of a reference ladder for alleles at locus D1S80. J Forensic Sci 1993;38:1176–82.
31. Cosso S, Reynolds R. Validation of the AmpliFLP™ D1S80 PCR amplification kit for forensic casework analysis according to TWGDAM guidelines. J Forensic Sci 1995;40:424–34.
32. Baechtel FS, Presley KW, Smerick JB. D1S80 typing of DNA from simulated forensic specimens. J Forensic Sci 1995;40:536–45.
33. Roffey PE. Separation of D1S80 alleles by vertical electrophoresis through a two-tier resolving gel. J Forensic Sci 1995;40:843–5.
34. Guidelines for a quality assurance program for DNA analysis. Crime Laboratory Digest, 1991;18:44–75.
35. Laber TL, Giese SA, Iverson JT, Liberty JA. Validation studies on the forensic analysis of restriction fragment length polymorphism (RFLP) on le agarose gels without ethidium bromide: Effects of contaminants, sunlight, and the electrophoresis of varying quantities of deoxyribonucleic acid (DNA). J Forensic Sci 1994;39:707–30.
36. Waye JS, Presley LA, Budowle B, Schutler GG, Fourney RM. A simple and sensitive method for quantifying human genomic DNA in forensic specimen extracts. Biotechniques 1989;7:852–5.

37. Carlson D, Horton M, Klevan L. An improved assay for quantitating human DNA. American Academy of Forensic Sciences 46th Annual Meeting; 1994 Feb 14–19; San Antonio (TX).
38. Walsh PS, Varlaro J, Reynolds R. A rapid chemiluminescent method for quantitation of human DNA. Nucl Acids Res 1992;20:5061–5.
39. Allen RC. Rapid isoelectric focusing and detection of nanogram amounts of proteins from body tissues and fluids. Electrophoresis 1980;1:32–7.
40. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC. Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 1991;48:137–44.
41. Darnay BG, Engel M. An efficient method for pouring sequencing gels. Biotechniques 1992;13:859.
42. Chang Y, Wu GE. A simple and rapid procedure for pouring sequencing gels. Biotechniques 1992;12:78.
43. Sambrook J, Fritsch JE, Maniatis T. Gel electrophoresis of DNA in "Molecular Cloning." In: Sambrook J, editor, 2nd ed., Cold Spring Harbor, NY, 1989;1:6.2–6.62.
44. Dykes DD. The use of biotinylated DNA probes in parentage testing: non-isotopic labeling and non-toxic extraction. Electrophoresis 1988;9:359–68.
45. Raymond M, Roussett F. Population genetics for exact tests and ecumenicism. J Heredity 1995;86:248–9.
46. Amplitype™ Users Guide, Version 2, Cetus Corporation, 1991.
47. Levene H. On a matching problem arising in genetics. Ann Math Stat 1949;20:91–4.
48. Hammond H, Caskey CT. Personal identification via short tandem repeats. In: The Third International Symposium on Human Identification 1992, Promega Corporation, 1992:163.

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