TECHNICAL NOTE

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Validation Studies of the CTT STR Multiplex System*

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ABSTRACT: Studies were performed to define the typing conditions and evaluate the forensic applicability of multiplex amplification of three STR loci, CSF1PO, TPOX, and THO1. Results were obtained using the GenePrint STR System (Promega Corporation, Madison, WI) Kit. To determine the utility of the GenePrint STR System for forensic casework analyses, the following experiments were conducted: 1) analysis of mixed body fluid; 2) determination of the sensitivity of detection; and 3) evaluation of results from biological samples from casework. In addition, the following simulated forensic conditions were assayed to detect whether or not there may be adverse effects on the ability to type these loci: 1) chemical contaminant effects on the DNA in body fluid samples; 2) the effects on DNA from samples deposited on various substrates; 3) the consequences of micro-organism contamination; and 4) the effect of sunlight and storage conditions on the integrity of the STR profiles/DNA. The data demonstrate that STR typing of biological samples exposed to a variety of environmental insults yields reliable results and that the analysis of the STR loci CSF1PO, TPOX, and THO1 can be applied in a forensic setting.

KEYWORDS: forensic science, DNA typing, genetic markers, validation studies, polymerase chain reaction, short tandem repeat loci, CSF1PO, TPOX, THO1, multiplex amplification

Short tandem repeat (STR) loci comprise a class of highly polymorphic loci in the human genome (1,2). STR loci are composed of tandemly repeated sequences that can be 2–7 base pairs in length, are small in size, generally less than 350 base pairs, and, thus, are amenable to amplification by the polymerase chain reaction (PCR) (3). Amplified products containing STRs can be resolved to at least the basic repeating unit by separation on denaturing polyacrylamide gels (1,2,4).

Lins et al. (5) described an analytical system comprised of three tetranucleotide repeat loci, THO1 (1,2,6), TPOX (7), and CSF1PO (8) which can be amplified simultaneously by PCR. The multiplex STR products were typed by electrophoretic separation in denaturing polyacrylamide gels and detection by silver staining. Because the sizes of the three STR loci in the multiplex do not overlap, operational designation of alleles is possible. Coamplification of the loci CSF1PO, TPOX, and THO1 (CTT) can be performed using a commercially-available kit (GenePrint Kit, Promega Corp., Madison, WI).

Before a technique can be used to analyze evidentiary samples, it should be evaluated for its forensic applicability. Although the effect of environmental insults on DNA is well-understood (9–11), the possible limitations of the use of a new test, such as STR loci typing, should be explored. This paper presents the results from a forensic validation study on the typing of the STR loci CTT.

Materials and Methods

Sample Preparation—The validation samples used in this study were a subset of those analyzed previously in D1S80 and Polymarker validation studies (9,10). For the validation study, body fluid samples from seven different donors were used. The STR triplex types of the donors are shown in Table 1.

The validation study consisted of the following analyses: 1) chemical contaminant effects on DNA; 2) the effect on typing DNA from body fluid samples deposited on various substrates; 3) the effect of micro-organism contamination on typing DNA derived from blood and semen; 4) the effect of sunlight and storage conditions on DNA typing (studies 1–4 are listed in Table 2); 5) detection sensitivity with mixed samples; 6) cross-reactivity of the CTT primers with DNA from micro-organisms, such as *Candida albicans, Staphylococcus epidermis, Bacillus subtilin,* and *Escherichia coli;* and 7) determination of the sensitivity of the fluorescent STR GenePrint Kit for CTTV (V is VWA locus) by typing genomic DNA from two samples at different quantities. The quantities of DNA amplified for these two samples ranged from 150 pg to 5 ng. In addition, samples from 52 adjudicated cases previously analyzed for the D1S80 locus were analyzed in this study. Also, some samples containing the THO1 9.3 and 10 alleles were analyzed.

DNA was extracted from the samples according to the method described by Comey et al. (12). The quantity of DNA in each sample was estimated using the chemiluminescent-based slot-blot procedure described by Waye et al. (13) and Budowle et al. (14). Generally, 0.5–5 ng of DNA were amplified by PCR.

Typing—The coamplification of the CTT loci was performed

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TABLE 1—*STR marker genotypes of body fluid donors for validation study.*

Donor	CSF1P0	TPOX	THO1
A	12,9	11,11	9.3,9
B	12,10	11,8	8,6
C	11,8	8,8	9,7
D	10,10	11,8	9.3,6
E	11,11	11,11	6,6
F	13,11	11,10	6,6
G	14,10	9,8	7,6

TABLE 2—*Results of environmental insult validation study.*

Specimen Substrate or Condition	Number of Samples Typed	Number Successfully Typed
Chemical Contaminant*/Blood	15	10†
Chemical Contaminant*/Semen	16	12†
Chemical Contaminant*/Saliva	16	12†
Substrate‡/Blood/Day 1	6	6
Substrate‡/Semen/Day 1	6	6
Substrate‡/Blood/Day 11	6	5§
Substrate‡/Semen/Day 11	6	4§
Substrate‡/Blood/Week 20	6	4§
Substrate‡/Semen/Week 20	6	6
Sunlight/Blood/Day 1-Week 20	12	12
Sunlight/Semen/Day 1-Week 20	12	6‖
Shade/Blood/Day 1-Week 20	12	12
Shade/Semen/Day 1-Week 20	12	12
Ambient Temperature/Blood/ Day 1-Week 20	12	12
Ambient Temperature/Semen/ Day 1-Week 20	12	12
Blood/C. Albicans	2	2
Semen/C. Albicans	2	2
Blood/S. Epidermidis	2	2
Semen/S. Epidermidis	2	2
Blood/E. Coli	2	2
Semen/E. Coli	2	2
Blood/B. Subtilis	2	1
Semen/B. Subtilis	2	2

*Chemicals are oil, gasoline, dirt, undiluted bleach, detergent, 0.4M NaOH, 5% Acetic Acid, 1M HCl.

†Negative results were with the chemical contaminants bleach and HCl; there was one negative result with oil and blood and one with dirt and blood.

‡Substrata were carpet, denim, leather, nylon, wallboard, and wood.

§Negative results were obtained for leather and semen (day 11), nylon and blood (day 11 and week 20), nylon and semen (day 11), and carpet and blood (week 20).

‖Negative results were for semen stains exposed to sunlight for 10 weeks or more.

¶The experiments were analyzed using Tris-Formate electrophoresis gels.

using the GenePrint STR System (Promega Corporation, Madison, WI) in 25 or 50 μL reaction volumes containing 0.5–5 ng template DNA, 2.5 units of Taq DNA polymerase per 50 μL reaction, and 8 μg of bovine serum albumin (Sigma, catalog #A3350) per 50 μL reaction (16). The primers for the STRs were described previously (15). Using the GeneAmp PCR System (GAPS) 9600, the PCR cycling parameters were denaturation at 95°C for 30 s, primer annealing at 67°C for 30 s, and primer extension at 70°C for 30 s, for a total of 28 or 30 cycles, depending on the initial quantity of template DNA. No mineral oil was placed in the tubes. Alternatively, the reactions (particularly CTT and VWA, also called "CTTV") were placed into a GAPS 9600 or a Perkin Elmer Thermal Cycler 480 and amplified according to the manufacturer's specifications: 96°C for 2 min, then 10 cycles at 94°C for 1 min, 64 or 60°C for 1 min, and 70°C for 1.5 min, and then 20 cycles at 90°C for 1 min, 64 or 60°C for 1 min, and 70°C for 1.5 min (and an extension for 30 min at 60°C, if the primers for the VWA locus are included in the PCR and the VWA locus is to be amplified). Kits are available with and without primer sets for the VWA locus. The tubes contained oil and for the GAPS 9600 the lid was not used.

Two different electrophoretic conditions were used to resolve the STR amplicons: continuous, denaturing polyacrylamide gels containing Tris-Borate-EDTA (TBE) (Method I) and discontinuous, denaturing polyacrylamide gels containing Tris-Formate and with Tris-Borate as the electrode buffer (Method II). Both methods utilized a sample loading buffer comprised of 10 mM NaOH, 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol FF. Both gel types were subjected to electrophoresis using a Model SA 32 Gel Electrophoresis System (Life Technologies, Gaithersburg, MD). Unlabeled amplicons were detected using silver stain (17), and fluor-labeled amplicons were detected using the FluorImager SI (Molecular Dynamics) with the photo multiplier tube set at 1000.

In Method I, 3 μL of loading buffer were mixed with 3 μL of PCR product. The samples were denatured at 95°C for 2 min, placed on ice, and then 5 μL were loaded onto the cathodal end of the continuous, denaturing gel (4%T, 5%C; cross-linker was bisacrylamide; 31 cm long and 0.4 mm thick; 7 M urea; 0.5X (or 90 mM) TBE, pH 8.3). The electrode reservoir contained 0.5X TBE. Electrophoresis was carried out at a constant power of 40 watts at ambient temperature, until the xylene cyanol dye migrated 6 cm from the anode (approximately 75 min).

In Method II, 3 μL of loading buffer were mixed with 3 μL of PCR product. The samples were denatured at 95°C for 2 min, placed on ice, and then 5 μL were loaded onto the cathodal end of the discontinuous, denaturing gel (6%T, 2%C; cross-linker was piperazine diacrylamide; 31 cm long and 0.4 mm thick; 7M urea, 60 mM Tris-formate, pH 9.0, with respect to the formate ion). The electrode buffer was 0.5X Tris-borate, pH 8.3. Electrophoresis was performed initially at 80 W for approximately 5 min and then continued with settings of 25 W at ambient temperature. The run was allowed to continue until the xylene cyanol tracking dye migrated to the top of the lower reservoir buffer (approximately 3 h).

Results and Discussion

Although different typing approaches were used in this study (i.e., PCR conditions, electrophoretic conditions, and detection approaches), there were no discrepancies in typing results. Typing results for the CTT multiplex were obtained using either the continuous or discontinuous denaturing gels and silver staining or fluor-detection using the FluorImager SI (Fig(s). 1 and 2).

Differences Between Silver Staining and Fluor Detection

With fluor detection, only one of the two strands from the denatured duplex can be observed; in contrast, silver staining enables detection of both strands (Fig. 1). With the denatured gel systems used in this study only the denatured strands at the THO1 locus are resolved. Nonetheless, detection of STR amplicons as single bands or as separated double bands does not compromise typing.

Fluor detection using the FluorImager SI was less laborious

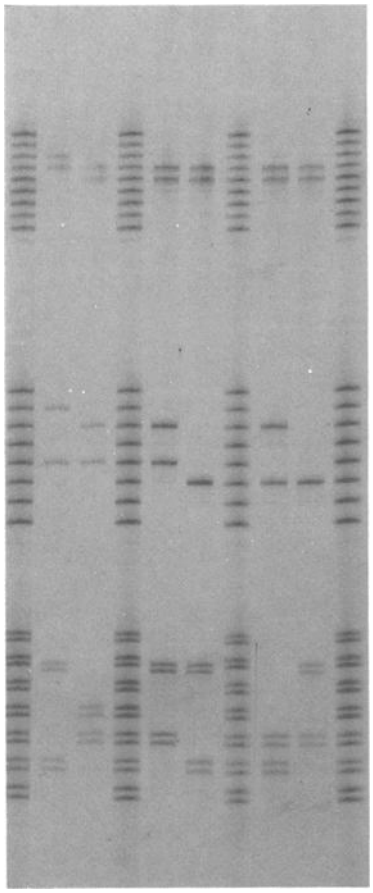

FIG. 1—*A silver-stained Tris-Formate gel displaying CTT profiles. The CSF1PO types from left-to-right are: allelic ladder, 13-12, 12-11, allelic ladder, 12-11, 12-11, allelic ladder, 12-11, 12-11, and allelic ladder. The TPOX types from left-to right are: allelic ladder, 12-9, 11-9, allelic ladder, 11-9, 8-8, allelic ladder, 11-8, 8-8, and allelic ladder; and the THO1 types from left-to-right are: allelic ladder, 9.3-6, 8-7, allelic ladder, 9.3-7, 9.3-6, allelic ladder, 7-6, 9.3-7, and allelic ladder. The cathode is at the top.*

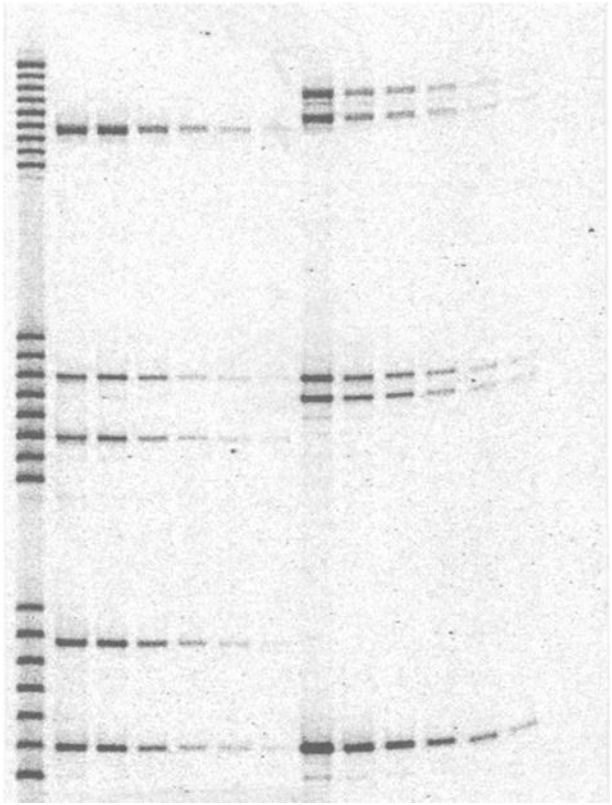

FIG. 2—*A Tris-Borate gel displaying fluor-labeled CTT products from two dilution series. Two samples were used. The types for the first sample are: CSF1PO—10-10, TPOX—11-8; and THO1—9.3-6; and the types for sample 2 are: CSF1PO—13-11, TPOX—11-10, and THO1—6-6. The template DNA used in each PCR dilution series from left-to-right is: 5, 2.5, 1.25, 0.6, 0.3, and 0.15 ng. The cathode is at the top.*

than silver staining. There was no need to separate the glass plates after electrophoresis; thus, there were no additional steps for staining, and potential tearing of gels was eliminated. In addition, there was no requirement for photography to record the image; the image can be stored in a computer and/or printed on an inexpensive paper medium. In contrast, silver staining does not require expensive equipment for detecting DNA.

The data support the contention that various methodological approaches can be used. The STR triplex typing procedures can differ, and reliable and consistent results can be obtained. Compared with RFLP analysis, which requires a substantial portion of the method to be standardized to obtain compatible results among laboratories, STR analysis allows more flexibility and protocol variation among users. Therefore, forensic laboratories can standardize on the result rather than on the method.

The Allele Sizes of the CTT Loci Are Discrete and Non-Overlapping

The alleles of the CTT differ sufficiently in size and mobility to enable each locus to be distinguished. The size range for each locus is: CSF1PO (295–327 bp), TPOX (232–248 bp), and THO1 (179–203 bp). Thus, alleles from different loci which have been amplified in a multiplex PCR can be assigned unambiguously to their respective loci. Nevertheless, the use of allelic ladders facilitates typing. This triplex can be used in DNA typing laboratories that are equipped with more expensive fluor detection apparatuses, as well as in laboratories with limited resources, where silver staining may be the only option.

All DNA fragments were separated electrophoretically in a denatured environment. The separation distances between fragments differing by one repeat unit were accentuated under denaturing conditions compared with native gel conditions (data not shown). With the formate-borate system, the approximate separation distance between CSF1PO alleles 7 and 15 was 1.5 cm, between TPOX alleles 8 and 12 was 1.2 cm, and between THO1 alleles 5 and 11 was 2.5 cm. All alleles in the triplex were resolved to one repeat unit (i.e., four base pairs). Moreover, the THO1 9.3 allele, which is a relatively common allele in Caucasians (1,2,6,18), is one base pair smaller in size than the 10 allele and could be resolved (Fig. 3). The ability to type unequivocally the THO1 9.3 allele (and separate the alleles contained in the allelic ladder) demonstrates that the electrophoretic system used in our study can resolve the common alleles in the CTT loci.

Stutter Bands

The CTT is composed of tetranulceotide repeat loci, which generally exhibit fewer stutter bands than loci containing tri- and

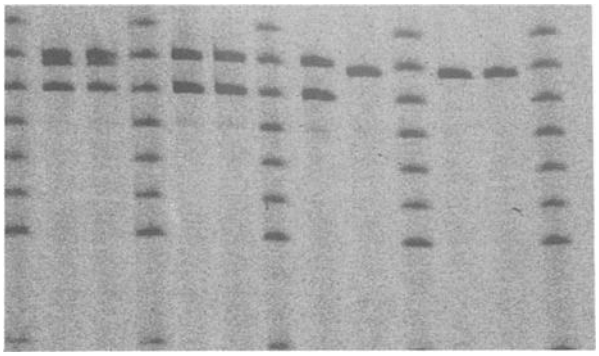

FIG. 3—*THO1 types demonstrating the resolving capacity of the electrophoretic system. The THO1 types from left-to-right are: allelic ladder, three alleles—10, 9.3, and 9, three alleles—10, 9.3, and 9, allelic ladder, 10-9, 10-9, allelic ladder, 10-9, 9.3-9.3, and allelic ladder. The cathode is at the top.*

dinucleotide repeats. The mechanism of stutter band formation may be due to slipped strand mispairing during the PCR (19) and generally migrate to positions one repeat smaller than the true allele (Fig. 4). With the conditions used in this study, the intensity of the stutter bands is relatively low compared with the true allele product (typically less than 10%), with the THO1 locus demonstrating the fewest amount of stutter products. The presence of stutter bands did not compromise interpretation in most situations in this study (see discussion on mixed samples below). Because most of the analyses in this study were performed using silver stain as the method of detection, densitometric analysis to determine the ratio of the quantity of stutter band to true allelic product was not performed. More studies are needed to determine whether or not the typically observed ratio of stutter band to allelic product can be useful for interpreting genotypes in mixed DNA samples.

Performance Parameters

Although the data demonstrate that STR loci typing of biological materials yields reliable results, particular observations were made regarding the performance of the STR loci typing system.

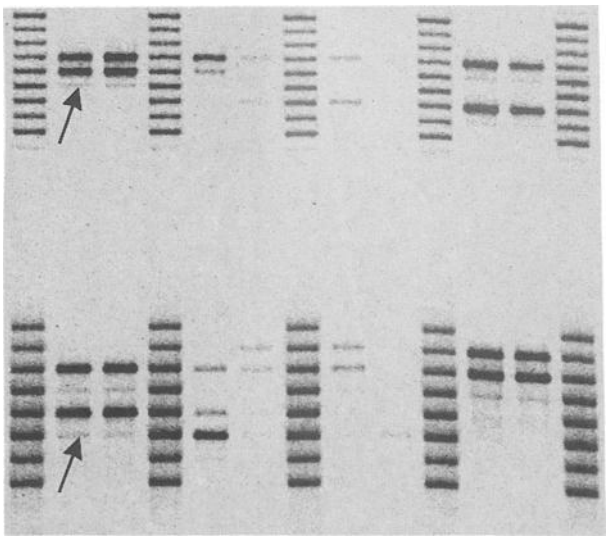

FIG. 4—*Examples of stutter bands at the CSF1PO and TPOX loci. The arrows indicate stutter bands that generally migrate to positions one repeat smaller than the true allele. The cathode is at the top.*

Depending on the particular kit format (i.e., triplex with the loci CTT or quadruplex with CTT and the STR locus VWA—CTTV), the manufacturer recommends a 64°C or 60°C, respectively, annealing temperature in the PCR. However, the CTT multiplex can be amplified under a higher stringency condition of 67°C without compromising amplification yield (Fig. 5). A PCR should be performed at the highest stringency possible to reduce potential mispriming events and thus minimize artifact products.

Amplification of the Geneprint Fluorescent CTTV quadruplex was carried out according the manufacturer's specifications using an annealing temperature of 60°C. A 64°C annealing temperature is noted by the manufacturer as "less desirable" due to poor performance of the CTTV system. Compared with the CTT triplex, the reduction in annealing temperature is necessary to accommodate amplification of the VWA locus, because the VWA primers apparently have a lower melting temperature than the CTT primers. In the current study, DNA from nine cases of sexual assault were analyzed with the Geneprint CTTV quadruplex using various PCR cycling conditions. In five out of nine cases, an extra band was detected in some samples using the recommended 60°C annealing temperature (Fig. 6). The artifact band migrated cathodal to the largest TPOX allele (allele 13) and did not correspond to an additional 4-bp repeat unit(s) (i.e., unlike a true allele). The artifact band was of greater intensity when the same quantity of template DNA (i.e., 5 ng) was amplified in the GAPS 9600 (Perkin-Elmer) compared with the DNA Thermal Cycler 480 (Perkin-Elmer). Moreover, the artifact band was more prevalent when the CTT multiplex was amplified in the GAPS 9600 (Perkin-Elmer) with no mineral oil overlaying the sample and the thermal cycler lid was used (in contrast to the manufacturer's recommended protocol for CTT typing). The artifact band was not detected when the CTT primers were annealed at 67°C and 64°C, but was visible at 60°C. As the annealing temperature was decreased to 57°C, the artifact band became more intense. This demonstration, in the absence of

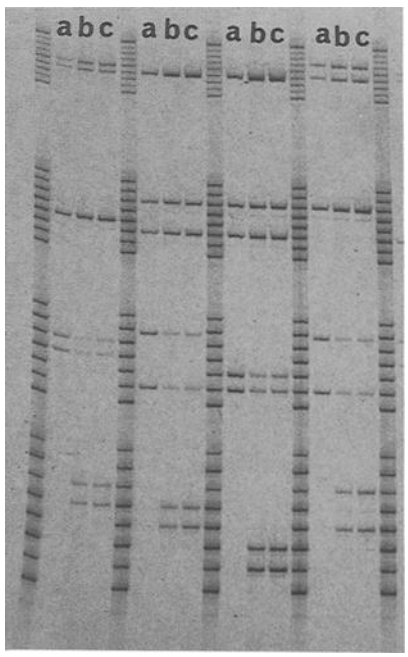

FIG. 5—*Effects of amplifying DNA for CTTV under a) our PCR conditions described in the Materials and Methods, b) manufacturer's protocol in a GAPS 9600, and c) manufacturer's protocol in a Perkin Elmer Thermal Cycler 480. The cathode is at the top.*

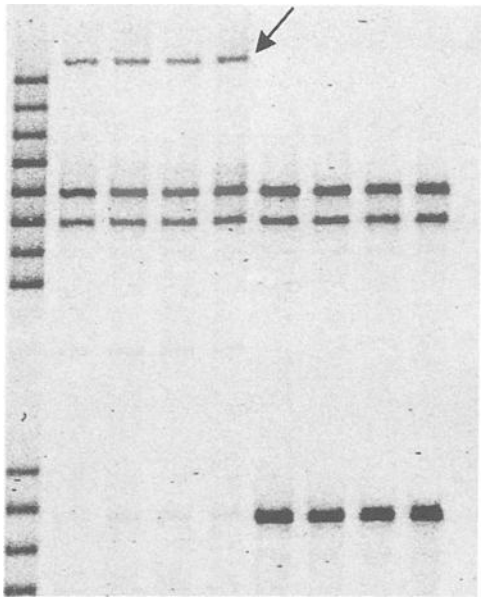

FIG. 6—*Example of artifact band residing cathodal to the TPOX locus, which is generated in a PCR with a 60°C annealing temperature. The arrow indicates the position of the artifact band.*

the VWA primers, indicates that the fragment is a non-specific product resulting from the reduction in annealing temperature, which occurs from either an inter-locus or intra-locus primer combination involving the CSF1PO, TPOX and/or THO1 loci. Although the artifact band can be eliminated by annealing at 64°C, amplification of the VWA locus is not achieved at this temperature.

A comparison of the quality of the PCR products from the CTTV multiplex under three different PCR cycling conditions is shown in Fig. 5. The two protocols for amplification are those recommended by the manufacturer for use with the GAPS 9600 (Perkin-Elmer) and the DNA Thermal Cycler 480 (Perkin-Elmer) (both with a 60°C annealing temperature and mineral oil overlaying the sample), and the third protocol is the one recommended herein for use with the GeneAmp PCR System 9600 (Perkin-Elmer) (i.e., 67°C annealing temperature and no oil in the PCR tube). The VWA locus requires an annealing temperature of 60°C and does not amplify at 67°C. However, at the annealing temperature of 60°C, the yield of THO1 locus product is reduced compared with the yield when amplified at 67°C. One plausible explanation for the reduction in THO1 product is, at 60°C, one of the THO1 primers or the template DNA might form a hairpin structure that prevents hybridization to template DNA (see the THO1 primer sequence in Huang, et al. (15)). A more balanced yield of products for all three CTT loci is obtained at an annealing temperature of 67°C.

The yield of THO1 may vary from lot-to-lot of the GenePrint STR kit. Reduction of the yield of THO1 product has been observed when a 60°C annealing temperature is used, even following the manufacturer's protocol for the GAPS 9600. Such variable performance characteristics and the potential for mispriming at lower stringency suggest that the conditions for PCR required to include the VWA locus compromise the quality of the CTT multiplex system. If it is desirable to add VWA as a fourth STR locus to the triplex, then the primers for the VWA locus should be redesigned to make the T_m (i.e., melting temperature) more similar to that of the other primers of the CTT system.

Sensitivity Study

Typing of the STR loci is possible with as little as 150–500 pg of genomic template DNA. However, there is no control, such as the C probe in the reverse dot blot system for HLA-DQA1 typing (20), for evaluating situations in which stochastic effects might occur due to amplifying too few copies of template DNA. To be consistent with the established stochastic parameters, the minimum amount of template DNA should be set at approximately 200–250 pg (the same level set for HLA-DQA1 typing). However, because a slot blot hybridization assay for quantitation of human DNA is a semi-quantitative assay (13,14), a minimum quantity of template greater than 200–250 pg is necessary to ensure that stochastic effects generally will not impact on STR profile interpretation. It is recommended that a minimum of 400–450 pg of template DNA be used for the PCR with the CTT system.

Environmental Insults

The reliability of typing DNA derived from environmentally-insulted samples is well-defined (9–11), and studies on additional or new classes of loci, such as STRs, add little to the knowledge of forensic utility and reliability of PCR-based DNA typing. However, some studies on environmental insults and their effects on STR loci typing were performed.

DNA extracted from blood, semen, and saliva exposed to a variety of environmental insults can be amplified by multiplex PCR and typed successfully at the STR loci CTT. These results are summarized in Table 2. Types were obtained for 151 of the 177 samples analyzed (a total of 85.3%). All typeable results were consistent with the known types of the donors.

Cross-reactivity of a DNA typing system with DNA from species other than humans (including higher primates) does not invalidate its use. However, for tests with forensic applications, it is important to determine whether or not DNA from other species can yield positive results. Previous studies (21,22) established that amplification of the CTT loci could only be achieved when using primate DNA. To supplement the cross-reactivity data, our study evaluated DNA from four micro-organisms (Table 2). No cross-reactivity was observed, and contamination with DNA from the micro-organisms did not impact on the ability to obtain reliable results from the human samples.

The environmental insult study described in Table 2 was performed using the silver stain-based detection method. A subset of these samples also was typed using the fluor-based detection method. Sixteen of 28 samples (a total of 57.1%) were typeable (data not shown), and typeable results were consistent with the known types of the donors of the samples.

Body Fluid Mixtures

The presence of two or more contributors to a sample generally is inferred by the presence of more than two bands at a locus or by differences in intensity of allelic bands within a locus profile. Figure 7 shows some examples of mixtures on CTT loci typing gels. In general, interpretation of mixtures is similar to that in other DNA typing systems. However, when DNA from one of the contributors is at a substantially greater level than another source in a mixed sample, the presence of stutter bands must be considered when interpreting mixtures (Fig. 7). Obviously, the type of the major contributor can be determined, but the minor contributor, depending on the particular profile, may be more problematic. In some instances, the minor contributor may be completely typeable;

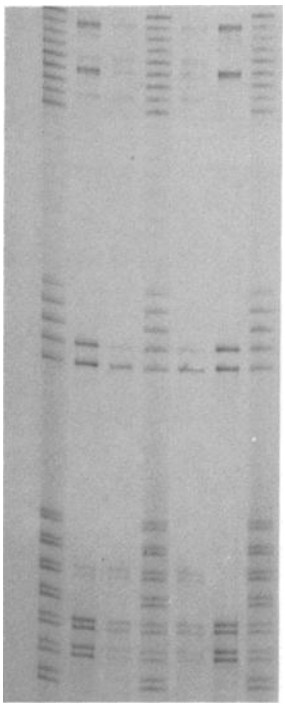

FIG. 7—*Some examples of CTT profile from mixed samples.*

in some instances, portions or the complete profile of the minor contributor may be masked by alleles or stutter bands from the major contributor and thus yield uninterpretable or inconclusive results for the minor contributor. In some situations, the data may be only useful for exclusionary purposes. Because of the myriad of possibilities for potential mixed samples, interpretation of results should be addressed on a case-by-case basis.

Case Work Study

A total of 52 cases was analyzed. Case samples were derived predominately from sexual assault cases, comprised of 114 known and 151 questioned samples. For the questioned samples 137 were from differential extractions (70 lysed or 'female fractions' and 67 non-lysed or 'male fractions') and 14 samples were from stains on various substrates. Of the 114 known samples, 108 were typeable (94.7%), and 115 out of 151 questioned samples (76.2%) were typeable. The number of typeable results is likely to be an underestimate of the performance of CTT typing because these samples are remnants of casework materials, i.e., the best portion of the sample was used for casework analysis. There were no discrepancies in the typeable results for the CTT loci and the data derived previously from the D1S80 locus (data not shown).

After the differential extraction, the swab or cutting that had been subjected to extraction was saved and subsequently extracted again by placing the sample into stain extraction buffer (containing dithiothreitol) followed by standard phenol extraction. Of these 69 samples, 60 re-extracted samples yielded sufficient DNA to obtain typeable profiles consistent with the male and/or female fractions. Interestingly, sometimes the female component DNA predominated and in other circumstances the male component was the major type. Thus, consideration should be given before disposing a swab or cutting after differential extraction.

The extracted DNA from samples from 9 of the 52 cases also was amplified and typed using fluor-based detection instead of silver stain. Out of a total of 68 samples, 60 samples were typed successfully (a total of 88.2%). The types obtained by silver and fluor detection were consistent.

Besides the amplification artifact discussed previously, there was another observation of extraneous bands. Figure 8 shows an example in which weak band patterns appeared in the area of a gel between the CSF1PO and TPOX loci. Although anodal to the CSF1PO locus profiles, these band patterns were similar to the CSF1PO profiles in the same sample lane. This observation is most likely the result of renaturation of some of the denatured CSF1PO strands. The presence of the renatured profiles did not affect profile interpretation and thus is not problematic. Although unnecessary, if it is desired, the sample can be denatured again and retyped.

Conclusion

Multiplex amplification by PCR of STR loci of DNA exposed to a variety of environmental insults yields reliable typing results. There was no evidence of false positive or false negative results. Generally, there was no evidence of preferential amplification within a locus, and usually when data were obtained at one locus, the other loci in the triplex also were typeable. However, it can be anticipated, that in some highly degraded samples, the smallest-size locus, i.e., THO1, may be typeable when the larger loci in the triplex may not be typeable.

Both continuous and discontinuous electrophoretic systems and silver and fluor detection methods provide reliable results. The data support that characterization of DNA derived from forensic biological materials using the CTT system is valid.

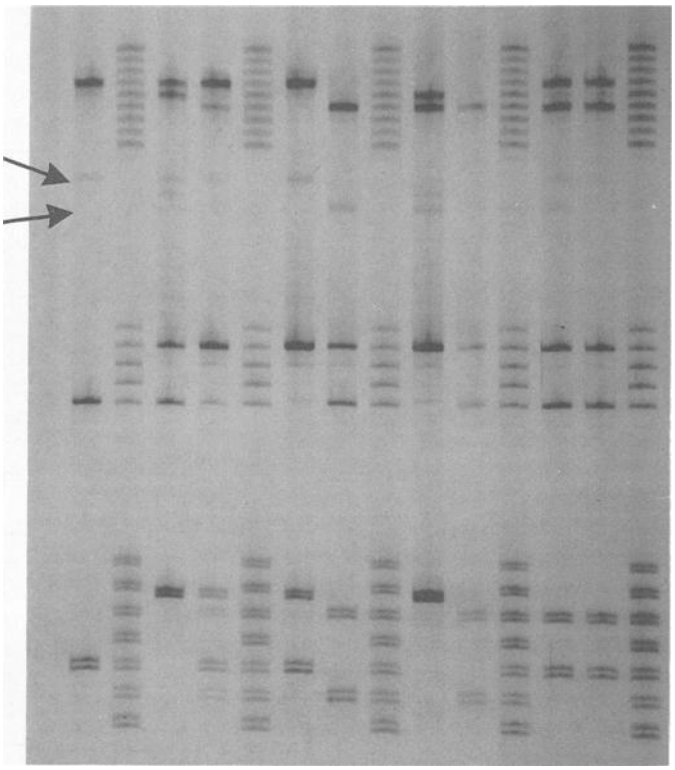

FIG. 8—*Example of extraneous bands anodal to the CSF1PO locus and cathodal to the TPOX locus. The arrows indicate the position of the extraneous bands.*

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