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Identification of Forensic Samples by Using an Infrared-based Automatic DNA Sequencer

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We have recently introduced a new protocol for analyzing all core loci of the Federal Bureau of Investigation's (FBI) Combined DNA Index System (CODIS) with an infrared (IR) automatic DNA sequencer (LI-COR 4200). The amplicons were labeled with forward oligonucleotide primers, covalently linked to a new infrared fluorescent molecule (IRDye™800). The alleles were displayed as familiar autoradiogram-like images with real-time detection. This protocol was employed for paternity testing, population studies, and identification of degraded forensic samples. We extensively analyzed some simulated forensic samples and mixed stains (blood, semen, saliva, bones, and fixed archival embedded tissues), comparing the results with donor samples. Sensitivity studies were also performed for the four multiplex systems. Our results show the efficiency, reliability, and accuracy of the IR system for the analysis of forensic samples. We also compared the efficiency of the multiplex protocol with ultraviolet (UV) technology. Paternity tests, undegraded DNA samples, and real forensic samples were analyzed with this approach based on IR technology and with UV-based automatic sequencers in combination with commercially-available kits. The comparability of the results with the widespread UV methods suggests that it is possible to exchange data between laboratories using the same core group of markers but different primer sets and detection methods.

Key words: *databases; DNA; DNA fingerprinting; forensic medicine ;polymerase chain reaction; tandem repeat sequences*

The standard for forensic genetics is based on the analysis of a selected panel of short tandem repeat (STR) systems in conjunction with the Amelogenin locus for sex determination. We recently reported the use of an infrared (IR) automated fluorescence monolaser sequencer (LI-COR-4200, LI-COR Inc., Lincoln, NE, USA) for the analysis of 13 autosomal STR systems (TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S 359, D18S51, and D21S11) and the X-Y homologous gene Amelogenin system (1). These systems are particularly important because they represent the core of the Combined DNA Index Systems (CODIS) used by the Federal Bureau of Investigation (FBI) in the United States (2) and include all markers used for DNA databases in Europe, except SE33 in Germany (3).

Some authors reported the possibility of using IR-labeled primers (4), commercial kits in combination with IR-labeled deoxyribonucleotide triphosphate (dNTP) (5), or primers with an IR/M13-tail (6,7) in forensic analysis. The instrument we employed was a LI-COR-4200 monolaser sequencer (LI-COR Inc.). This real-time detection system combines high-sensitivity infrared fluorescence chemistry and laser technology and permits immediate visualization of the raw data. The alleles are displayed as autoradiogram-like images, which can be analyzed by software included in the computer. The near-infrared region of the spectrum provides distinct advantages, because the fluorescence emission of solvents, biomolecules, and glass is minimal. The extremely low background and high sensitivity allow detection of diminutive quantities of labeled molecules (8). However, it was necessary to design multiplex systems with non-overlapping amplified products, because the microscope of this system is able to detect only a single wavelength.

Our protocol was based on four independent multiplex polymerase chain reactions (PCR): two tetraplex systems (MU1: AMELOGENIN, vWA, FGA, and D16S359; and MU2: D3S1358, TPOX, TH01, and CSF1PO), and two triplex systems (MU3: D8S1179, D21S11, and D18S51; and MU4: D7S820, D5S818, and D13S317). The forward primer of every primer pair was labeled with a new fluorochrome

(IRDye™800, LI-COR Inc.). This protocol was used to solve paternity testing and collect data in a Tuscan population of 188 unrelated individuals (9).

In a preliminary study, we investigated the efficiency of this protocol for the analysis of forensic samples (1). However, for degraded samples and forensic samples collected in normal casework, additional studies were required. Thus, to verify the efficiency of the IR technology in combination with IRDye™800-labeled oligonucleotide, we analyzed a large number of various kinds of forensic samples. A sensitivity study was also performed. Furthermore, to improve the efficiency of the method, we slightly modified the original protocol. PCR conditions were optimized by increasing the amplification cycles to 34. Moreover, a different PCR strategy was used to amplify the four STRs of MU2. Finally, a study was performed in several laboratories. Some degraded and undegraded forensic samples were exchanged between laboratories using the same STR core loci but employing different labeling and detection technologies. The results obtained were completely in agreement for the undegraded samples and for the particularly difficult forensic samples, although there were a few minor differences in the number of successfully typed samples.

Material and Methods

DNA Samples

A total of 42 DNA samples were examined. Five cigarette butts, six stamps, five hairs, three semen samples, and 12 bloodstains were obtained from known donors. The samples had been stored between 1 month and 3 years at room temperature before processing. Five archival formalin-fixed tissues and six histologic sections were also compared with patients' blood. Moreover, the alleged father's bones that were ten years old were available for paternity testing.

Sensitivity Study

We used either serial dilutions of K562 control DNA (Promega Corporation, Madison, WI, USA) or genomic DNA from 10 ng/μL to 5 pg/μL. The amount of amplified DNA was 5 ng, 2 ng, 500 pg, 100 pg, 50 pg, 25 pg, 10 pg, and 5 pg, as the template for each amplification reaction. In the second approach, genomic DNA from a known woman and a known man were mixed and amplified together. The female/male DNA ratio in 0.9 μL used for PCR ranged from 1 ng : 1 ng to 1 ng : 0.05 ng (1:1-1:20).

Interlaboratory Study

Samples previously typed in other laboratories using UV technology and commercially-available kits were referred for blind analysis to the laboratory using IR technology. Subsequently, the results were exchanged and compared. The samples consisted of 10 undegraded DNA samples from unrelated individuals; genomic DNA from four paternity tests involving 17 persons, in which a single mutation event was observed; and 28 real forensic samples from various sources (blood, semen, and saliva).

DNA Extraction

The simulated forensic samples were extracted with Chelex (10) or alkaline lysis method (11). Archival formalin-fixed tissue and histologic sections were extracted according to the procedure described by Coombs (12). DNA was extracted from bone with phenol chloroform. From donors' blood DNA was obtained by using a Puregene® DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). All forensic samples for the interlaboratory study were extracted from the various sources with the QIAamp® tissue kit (Quiagen, Hilden, Germany).

Typing

UV-typing. Undegraded DNA and forensic DNA samples for the comparative analysis were typed by using the AmpF*l*STR® SGM Plus™, AmpF*l*STR® Cofiler™, and AmpF*l*STR® Profiler Plus™ PCR Amplification kits, in combination with the ABI PRISM® 310 Genetic Analyser (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's recommendations. For the paternity tests, 1-20 ng of genomic purified DNA were used, and 5 μL of non-quantified extract were used for the analysis of forensic samples according to the previously described protocol (13).

Table 1. Characteristics of the loci investigated: chromosomal location, GenBank accession number, primer sequence, size, repeat sequence, genotype of K562 cell line, and references

Locus	Chromosomal location	GenBank accession	Primer sequence	Alleles size (bp)	Repeat sequence	K562 DNA	Ref. No.
TPOX	2p23-2pter	M68651	A - ACTGGCACAGAACAGGCACTTAGG B - GGAGGAACTGGGAACCACACAGGT	232-248	(AATG)n	8, 9	14
D3S1358	3p	196594	A - ACTGCAGTCCAATCTGGGT B - ATGAAATCAACAGAGGCTTG	123-135	TCTA (TCTG)1-3 (TCTA)n	16, 16	15
FGA	4q28	M64982	A - GCCCCATAGGTTTTGAACTCA B - TGATTTGTCTGTAATTGCCAGC	176-224	(TTTC)3 TTTT TTCT (CTTT)n CTCC (TTCC)2	21, 24	16
CSF1PO	5q33.3-34	X14720	A - AACCTGAGTCTGCCAAGGACTAGC B - TTCCACACACCACTGGCCATCTTC	299-323	(AGAT)n	9, 10	17
D5S818	5q23.3-32	G08446	A - GGGTGATTTTCCTCTTTGGT B - TGATTCCAATCATAGCCACA	149-161	(AGAT)n	11, 12	18
D7S820	7q	G08616	A - TGTCATAGTTTAGAACGAACTAACG B - CTGAGGTATCAAAAACTCAGAGG	198-222	(GATA)n	9, 11	18
D8S1179	8q24.21	AF250877	A - TTTTTGTATTTCATGTGTACATTCG B - CGTATCCCATTGCGTGAATATG	127-163	(TCTR)n with R = A or G	12, 12	1
TH01	11p15-15.5	D00269	A - GTGGGCTGAAAAGCTCCCGATTAT B - GTGATTCCCATTGGCCTGTTCCTC	154-178	(AATG)n	9.3, 9.3	19
vWA	12p12-pter	M25858	A - CCCTAGTGGATGATAAGAATAATCAGTATG B - GGACAGATGATAAATACATAGGATGGATGG	134-162	TCTA (TCTG)3-4 (TCTA)n	16, 16	20
D13S317	13q22-31	AF250876	A - GTTGCTGGACATGGTATCACAG B - TCAGAGAGCTTGAATTGTTGGT	245-261	(GATA)n	8, 8	1
D16S359	16q22-24	AF249681	A - ACTCTCAGTCCTGCCGAGGT B - TGTGTGTGCATCTGTAAGCATG	350-370	(AGAT)n	11, 12	1
D18S51	18q21.3	L18333	A - CAAACCCGACTACCAGCAAC B - GAGCCATGTTCATGCCACTG	274-318	(AGAA)n	15, 16	21
D21S11	21q11.2-q21	M84567	A - ATATGTGAGTCAATTCCCCAAG B - TGTATTAGTCAATGTTCTCCAG	214-240	(TCTA)n (TCTG)n [(TCTA)3 TA(TCTA)3 TCA (TCTA)2 TCCA TA] (TCTA)n	29,30,31	22
HUMAMGXA HUMAMGY	Xp22.1-p22.3 Yp11.2	M86932 M86933	A - CCCTGGGCTCTGTAAAGAATAGTG B - ATCAGAGCTTAAACTGGGAAGCTG	106-112		X - X	23

IR-typing. Table 1 shows the primer sequences of the primers used, and Table 2 shows the final primer concentrations. Here, a different PCR strategy was adopted to amplify the four loci of the MU2 system (D3S1358, TPOX, TH01, and CSF1PO) by using just a small amount of each forward-labeled primer in a mixture containing forward and reverse unlabeled primers.

Table 2. Primer concentrations of the multiplex polymerase chain reaction (PCR) amplification systems

System	Locus	5'IRDye™800 Forward primer (μmol/L)	Forward primer (μmol/L)	Reverse primer (μmol/L)
MU1	amelogenin	0.40	–	0.40
	vWA	0.25	–	0.25
	FGA	0.10	–	0.10
	D16S359	0.20	–	0.20
MU2	D3S1358	0.01	0.14	0.14
	TPOX	0.02	0.20	0.20
	TH01	0.02	0.20	0.20
	CSF1PO	0.08	0.40	0.40
MU3	D8S1179	0.60	–	0.60
	D21S11	0.30	–	0.30
	D18S51	0.10	–	0.10
MU4	D7S820	0.20	–	0.20
	D5S818	0.20	–	0.20
	D13S317	0.30	–	0.30

The amplifications were performed in a total volume of 12.5 μL with 1-3 ng of genomic DNA in GeneAmp® PCR buffer II (Applied Biosystems) containing 100 mmol/L Tris-HCl (pH 8.3) and 500 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 mmol/L of dNTP, and 0.3 U of AmpliTaq Gold™ (Applied Biosystems). Stock solutions A and B were prepared for each multiplex system. Solution A contained buffer, magnesium, dNTPs, polymerase, and bidistilled water. Solution B contained labeled and unlabeled primers and bidistilled water. A mixture of 5.8 μL of solution A and 5.8 μL of solution B was prepared and 0.9 μL of DNA was added before PCR. When forensic DNA samples gave negative results, 0.9 μL of a 1:10 dilution was used for PCR. The following amplification conditions were used: for MU1, MU2, and MU4, 95 °C for 10 minutes, then 94 °C for 1 minute, 60 °C for 1 minute, 72 °C for 1 minute, for 34 cycles, with final extension at 72 °C for 60 minutes. For MU3, the annealing temperature was 58 °C.

At the end of the amplification cycles, 6 μL stop solution containing 95% formamide, 10 mmol/L ethylenediaminetetraacetic acid (EDTA) and 0.1% blue bromophenol were added. Gel electrophoresis was performed in a LI-COR®-4200 monolaser automated fluorescent DNA sequencer by using gel 33 cm long and 2.5 mm thick. The used sequencing gel solution was SequaGel® XR Extended Range Gel Solution for DNA Electrophoresis (National Diagnostics, Atlanta, GA, USA) containing 6 mol/L urea in running Tris-borate-EDTA buffer 1× (89 mmol/L Tris base, 89 mmol/L boric acid, and 2 mmol/L EDTA). The gels were run at a constant power of 31.5W, initially at 950 V and 35 mA (maximum 1550 V), at a constant temperature of 50±1 °C. A 48 sharkstooth comb was used for the well construction. After each sample was denatured for 2 min at 94 °C, 2 μL were loaded with a Hamilton syringe. An allelic ladder was loaded every eight samples, and two positive control samples were loaded on every gel. The image file was completed 2-3 h after sample loading, depending on the size of the fragments.

The result of the run appeared in an autoradiogram-like image, automatically recorded as a TIF image and filed on the computer hard disk. Alternatively, the data could be observed as a typical electropherogram (Fig. 1). The bands present at various positions in the gel virtual image were recorded as a signal of fluorescent intensity on the basis of their transit time through the detector. The patterns detected by the automated sequencer could be conveniently managed and analyzed by using the "Base ImageIR 3.0" software, which allowed each single multiplex system to be examined separately (8). The exact identification of the alleles was carried out by using ladders constructed with sequenced alleles, used in previous Italian and European collaborative projects (1,24). As the positive control sample, K562 control DNA cell lines were used. The classification of the alleles was in agreement with the recommendations of the International Society for Forensic Genetics (ISFG) Commission (2). By using the software, a quantitative assay of band areas was also possible, this being particularly useful in the analysis of mixed stains. The electropherogram documentation was achieved with the "Base ImagIR™ Manipulation" program, which allowed manipulation of the image files in a variety of ways. The image could be cropped so that only a portion of it was printed, with enhancements and/or resized printing, saving in alternate file formats (TIFF or EPS), sizing, and scaling.

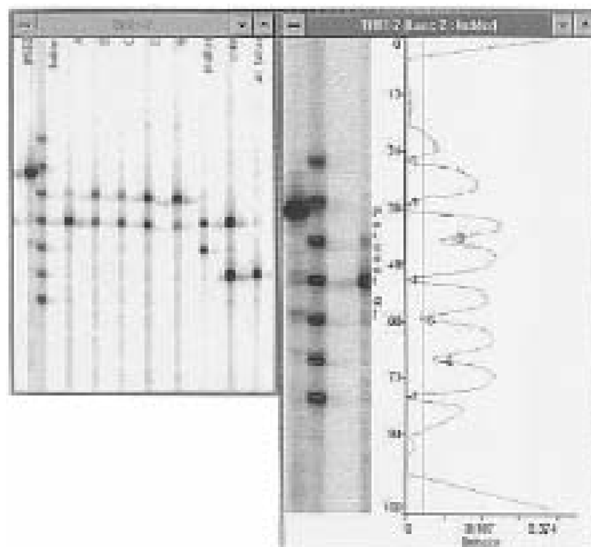

Figure 1. An example of an autoradiogram-like image of locus TH01 (left) and the quantitative peak profile of the allelic ladder (right).

Results

The purpose of this research was to evaluate the potential of an IR protocol for the analysis of forensic samples. The profiles were obtained with the four multiplex systems by analyzing some forensic samples from known individuals and using control DNA and allelic ladders (Fig. 2).

Amplification Conditions

Primer concentrations in the four independent amplification systems were established to obtain balanced signals. We used AmpliTaq Gold™ Polymerase (Applied Biosystems) to amplify the DNA target with an extended pre-incubation of 95 °C for 10 minutes to enhance yield and specificity (26). As reported previously (27), when <100 pg of DNA was analyzed, there was no advantage in using more than 34 cycles, because allele dropout due to stochastic variation may always occur. For this reason, 34 PCR cycles were systematically used to amplify the forensic samples. We observed that, when the template DNA was excessively concentrated, the intensity of the signal increased proportionately for all loci and an extra band of minor intensity could be seen for all alleles (Fig. 2). In these cases, we were able to reanalyze diluted samples. Moreover, the acquisition software was able to modify the sensitivity of the image using a 16-bit format to collect data (more data per pixel), and

reduction/enhancement of the signal was always possible. In our experience, over-amplification of forensic samples using this protocol is rare and the correct profile can always be obtained.

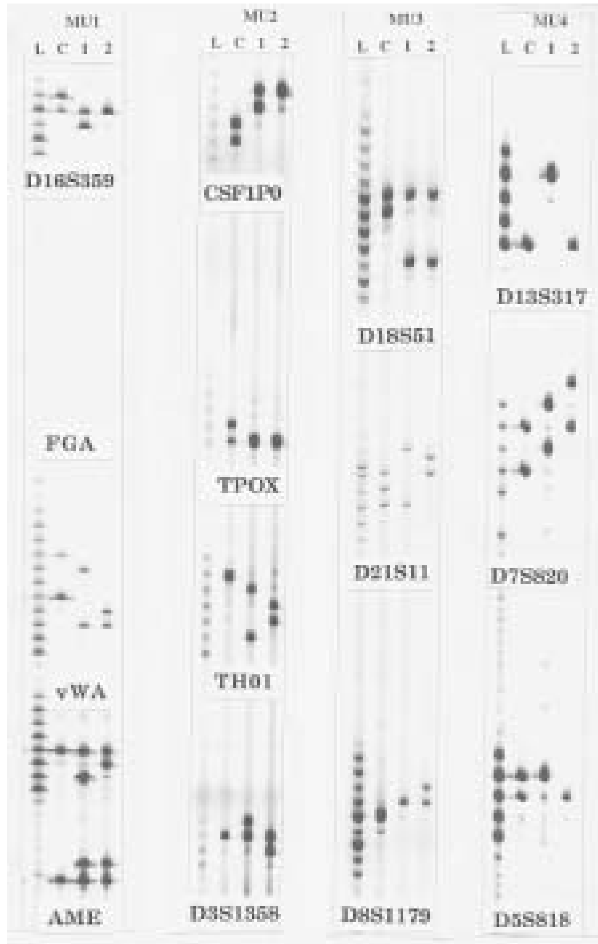

Figure 2. Examples of genetic profiles of the 13 CODIS systems plus the locus of the Amelogenin for sex determination, obtained with the 4 multiplex systems (MU1-4) analyzing archivial paraffin-embedded samples from two known individuals (lane 1 and 2). L – allelic ladder; C – K562 cell lines. The documentation was created by means of the "Base ImagIR™ Manipulation" program, selecting each multiplex independently.

Stock solutions A and B for each multiplex system, stored at 4 °C away from the light, are usually stable for four months.

Typing

Amelogenin, D3S1358, TH01, TPOX, CSF1PO, D8S1179, and D18S51 showed a double-band pattern (Fig. 2). The final extension period introduced at the end of the PCR reactions was not sufficient to significantly shift the bias in favor of an extra base addition at every locus, like reported previously (16). However, it does not present a problem for the correct identification of the alleles by means of comparison with the reference ladders. Occasional "shadow bands" observed between loci can be disregarded as being out of the amplification range of the multiplex systems.

The artifacts and stutters in MU2 amplification system were extremely reduced, compared with the results obtained with only forward labeled and reverse unlabeled primers (data not shown).

In this study, 42 forensic samples, including cigarette butts, stamps, semen, bloodstains, archival formalin-fixed tissues, and histologic sections, were all successfully typed in comparison with the donor blood. All analyses showed identical allelic profiles in each individual for a wide variety of different samples typed. In a few cases, we obtained no results for D16S359, the largest locus in our protocol (350-370 bp). No allelic dropout was observed. Amelogenin, FGA, D5S818, and D18S51 gave the best results. Various forensic samples (bloodstain, hair, saliva, and sperm) were compared with DNA donor (Fig. 3).

A paternity test in which ten years old bones from the alleged father were obtained after exhumation showed compatibility for all 13 STR markers (probability of paternity, W>99.99%).

Sensitivity Study

The results we obtained using serial dilutions of K562 DNA or genomic DNA in sterile bidistilled water indicated that up to 10 pg of undegraded DNA could be detected for each of the loci of the MU1, MU3, and MU4 systems by this protocol. The MU2

Table 3. Results of comparative analysis between ultraviolet (UV)-technology and infrared (IR)-technology for 28 very degraded forensic samples from various sources analyzed in the present study*

	Sample No.																											
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
TPOX	‡	‡	IR UV	†	‡	†	†	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	†	‡	†	†	IR	†	‡	†	‡
D3S1358	‡	‡	IR UV	†	‡	UV	IR	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	IR	‡	†	†	IR	†	‡	†	‡
FGA	‡	‡	IR UV	†	‡	†	IR	‡	†	‡	‡	‡	‡	‡	‡	IR	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
CSF1PO	‡	‡	UV	†	‡	†	†	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	†	†	IR	‡	†	†	IR	†	‡	†	‡
D5S818	‡	‡	IR UV	IR	‡	†	†	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
D7S820	‡	‡	IR UV	†	‡	†	IR	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
D8S1179	‡	‡	UV	†	‡	†	IR	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
TH01	‡	‡	UV	†	‡	†	IR	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
VWA	‡	‡	IR UV	IR	‡	UV	IR	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
D13S317	‡	‡	UV	IR	‡	†	†	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
D16S359	‡	‡	UV	†	‡	†	†	‡	†	‡	‡	‡	‡	‡	‡	†	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
D18S51	‡	‡	UV	IR	‡	†	†	‡	†	‡	‡	‡	‡	‡	‡	IR	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡
D21S11	‡	‡	IR UV	†	‡	†	†	‡	†	‡	‡	‡	‡	‡	‡	†	‡	IR	†	†	‡	†	†	†	†	‡	†	‡
AME	‡	‡	IR UV	IR	‡	UV	IR	‡	†	‡	‡	‡	‡	‡	‡	IR UV	‡	IR	†	IR	‡	†	†	†	†	‡	†	‡

*Abbreviations and designations: † – no result; IR – result obtained only with the infrared method; UV –result obtained only with ultraviolet method; ‡ – result obtained with both methods; AME – amelogenin.

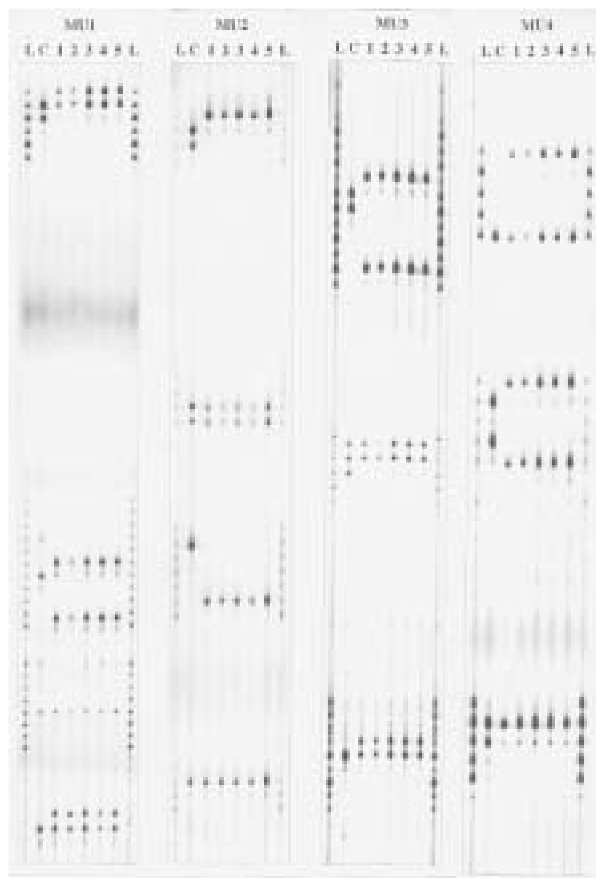

Figure 3. Comparison of various forensic samples with DNA donor. Lane 1 – six-month-old sperm stain; lane 2 – freshly-plucked hair; lane 3 - one-month-old bloodstain on fabric; lane 4 – saliva from a cigarette butt; lane 5 – genomic DNA; L - allelic ladder; C – K562 cell lines. All analyses show identical allelic profiles in each individual for a wide variety of different samples typed.

amplification system was particularly efficient in the analysis of a reduced amount of DNA template. In the sensitivity study where serial dilutions of DNA were used, we observed positive results when 5 pg of undegraded DNA was used (Fig. 4). Since it is difficult to accurately quantify low levels of DNA, we estimated the amount of DNA at <10 pg. Full profiles from mixed genomic female and male DNA up to a 1:12 ratio were obtained for all systems as well as the Amelogenin locus. Using the Calc Integrated Density option in the "Base Image IR 3.00" software, we were able to compute integrated densities for all bands in the specified lane. We also verified the efficiency of the systems in two experiments in which semen and blood were mixed together.

Interlaboratory Study

The interlaboratory evaluation gave consistent results. Twenty-seven undegraded samples (10 unrelated individuals and 17 persons involved in three paternity tests) were typed and identical results obtained by all three laboratories included in the study. The single mutation event in each paternity test was confirmed afterwards by independent laboratories.

Figure 4. Sensitivity study for the MU2 amplification system, using serial dilutions of DNA. L – allelic ladder; C – K562 cell line; lanes 1-8 – serial dilutions of DNA: 5 ng, 2 ng, 500 pg, 100 pg, 50 pg, 25 pg, 10 pg, and 5 pg.

The analysis of the 28 real forensic samples exchanged between the laboratories showed consistent results (Table 3), with 71% of the samples in complete agreement (14/28 full profiles, and 6/28 fully negative). In two samples, positive results were obtained with a 1:10 dilution before amplification. For the remaining 29% (8/28) of the samples, both laboratories obtained only incomplete profiles. For these samples the successfully typed loci varied, but in general, most problems arose with the largest loci (D18S51, FGA, and D16S359 for the UV protocol; D16S359, D18S51, and D13S317 for the IR protocol).

Previous results indicate that the IR fluorescence technology is approximately 3-50 times more sensitive than visible fluorescence (28). However, our results in real forensic casework did not seem to support significant differences, and the minor variation in typing seemed to occur because of a random fluctuation (pipetting or dilution). Specific studies using identical PCR conditions will be necessary to compare the sensitivity of the IR and UV systems.

Discussion

Amplification of the STR loci for forensic purposes is routinely performed by using UV, automated DNA sequencers, and commercially-available kits. According to validation studies, publications, and the experience of many laboratories, we think there is no reason for changing the technology. Use of an IR-based automated sequencer in forensic genetic laboratories has become less frequent. The lack of interest may result from the need to perform four separate amplifications of a sample in order to obtain the same amount of data that one or two amplifications with other types of fluorescent detection instrumentation would provide. However, the infrared technology is reliable, the instrument is robust, and the laser has a long life (100,000 h) (8). Moreover, infrared fluorescence is extremely low due to the glass, solvents, and biomolecules, thus producing a very low background signal and high sensitivity (29). In our opinion, this protocol can be used for the analysis of the CODIS systems by the laboratories opting for the IR technology. Our report supports the efficiency of the four multiplex amplification systems in combination with an IR automatic DNA monolaser sequencer for the investigation of forensic samples. The test is reliable, accurate, and efficient and provides a highly-integrated package for high throughput for forensic analysis, while keeping the cost of automation affordable. The gels can be run up to three times and throughput can be increased by using 64-well combs. As the need arises, various samples for the same multiplex or the same samples for different multiplexes can be analyzed together. The use of the new generation of IR automated sequencers, which use a double laser, are able to detect two fluorochromes, and rely on the second-generation software "SAGA^{GT}" for genotype analysis, will enhance the throughput using IR technology. The multiplex systems we used did not overlap in size. Thus, it will be possible to use these systems for manual typing with polyacrylamide gel electrophoresis, and for the automatic sequencers that employ just one fluorochrome.

The comparison with the widespread UV-based DNA technology confirms that the two methods are comparably efficient in typing forensic stains. The consistent results observed in this study and practical casework so far suggest that it is possible to exchange data between laboratories using the same core of markers but different technologies.

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