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Analysis of Human Fecal Material for Autosomal and Y Chromosome STRs*

ABSTRACT: Human stool samples from eight volunteers were stored under various conditions and extracted by three different procedures. Fecal material and tissue paper soiled with fecal material obtained from a crime scene were also extracted. Extracted DNA was amplified using the AmpFℓSTR® Profiler Plus™, AmpFℓSTR® COfiler™, and the AmpFℓSTR® Identifiler™ PCR amplification kits for the detection of the autosomal STR allelic patterns. DNA extracted from the male volunteers and from the soiled tissue paper evidence sample was also amplified using the Y-PLEX™6 and Y-PLEX™5 amplification kits. Analysis of the amplified products was carried out by capillary electrophoresis on the ABI PRISM® 310 Genetic Analyzer. Autosomal and Y-STR profiles obtained from the fecal material were concordant with the results from the donors' buccal swabs.

KEYWORDS: forensic science, DNA typing, fecal material, polymerase chain reaction, short tandem repeats, DYS393, DYS19, DYS389II, DYS390, DYS391, DYS385, DYS389I, DYS439, DYS438, DYS392, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, amelogenin

Fecal samples, tissue paper smeared with fecal material and clothing containing human feces, may be the only evidence left at a crime scene. Thus, human stool can be a valuable source for forensic DNA evidence. The genetic markers of the submitted stool evidence can be used to determine the origin of such samples.

However, due to microbial presence in the gastrointestinal tracts, extraction of human DNA from feces may be challenging (1). Furthermore, stool contains high levels of compounds that can degrade DNA and inhibit downstream PCR reactions, and these compounds must be removed for reliable analysis of extracted DNA. For these reasons, conventional organic extraction may not always be useful in extracting DNA free of PCR inhibitors (2,3).

Occasionally, in the St. Louis County Police Crime Laboratory, cases are submitted where fecal matter, tissue paper, or clothing smeared with feces are seized by the investigators during the search for evidence.

The author conducted a validation study using human stool samples from eight volunteer donors. Buccal swabs were collected from these donors to obtain their autosomal short tandem repeat (STR) profiles. Y-STR profiles of the male donors were also obtained from their buccal swabs.

Material and Methods

The investigators seized fecal samples during the search of a robbery crime scene. The fecal matter evidence was submitted in the form of a solid black lump packaged in a plastic bag. Toilet tissue papers stained with fecal matter packaged in paper bags were also submitted in this case. Once received in the laboratory, both of these samples were stored at −20°C until extracted approximately six to eight weeks later. The environmental condition to which these samples were subjected prior to their seizure at the crime scene is unknown.

Four male and four female volunteers donated fecal material for this study. Each individual stored his or her sample at room temperature for one day in a closed plastic container. The containers were opened next day and left open. These opened containers were stored inside sealed paper bags at room temperature and extracted approximately eight weeks later. Portions of these samples were stored at −20°C after storage at room temperature for one day, and the frozen samples were extracted approximately eight weeks later. Samples from three male and four female volunteers were also smeared on toilet tissue paper (generic brand) immediately after defecation. A spatula was used to carefully scrape the outer edges of the stool, and the specimens were smeared on the paper. The tissue papers were stored at room temperature for approximately eight weeks prior to extraction.

DNA Extraction

The fecal materials collected from eight volunteers and stored at room temperature or at −20°C were extracted by three different extraction procedures. Since a greater number of epithelial cells are expected to be present on the outer edges of fecal matters, samples were scraped from the outer edges of the stool and the scrapings were weighed. The samples from four females and three male volunteers smeared on tissue papers were cut out, weighed, and extracted along with any tissue paper that might have adhered to the fecal matter by the three extraction procedures.

The organic extraction procedure using phenol/chloroform/isoamyl alcohol extraction (4) was used for extraction of DNA from volunteer fecal samples. Approximately a 250-mg sample from each volunteer was used for each sample tube. DNA from the organic extractions was subjected to a further purification step us-

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ing the Rapid PCR Purification System from Marligen Bioscience Inc. (Ijamesville, MD) as described previously for the PCR Concert™ Rapid Purification System from GIBCO-BRL (5).

All volunteer samples were also extracted by the two following procedures: QIAamp® DNA Stool Mini Kit and the UltraClean™ Fecal DNA Isolation Kit. Approximately a 220-mg sample was used for each extraction tube when extracted by the QIAamp® DNA Stool Mini Kit, and approximately a 250-mg sample of fecal matter was used for each bead tube when extracted with the UltraClean™ Fecal DNA Isolation Kit. Extraction of human stool was performed as per each manufacturer's recommendations (6,7). DNA obtained from each tube processed by the same procedure was combined into a single tube, and this pooled DNA was concentrated further by using Microcon® 100 devices (Millipore Corporation, Bedford, MA).

The solid fecal matters from the crime scene were subjected to all of the three extraction procedures described above. The tissue papers soiled with fecal samples seized at the same robbery crime scene were extracted using the organic extraction procedure and by the QIAamp® DNA Stool Mini Kit from QIAGEN Inc. (Valencia, CA). Due to limited sample size, the fecal matters on the tissue papers obtained from the crime scene were not processed by the UltraClean™ Fecal DNA Isolation Kit from MO BIO Laboratories, Inc. (Solana Beach, CA). The DNA from the solid fecal matter or the soiled tissue paper from the robbery crime scene was not subjected to the Rapid PCR Purification System after organic extraction.

All buccal swabs were stored at −20°C and extracted by organic extraction approximately eight weeks later. Extracted DNA samples were purified with the Rapid PCR Purification System.

DNA Quantitation

Extracted DNA was quantitated using a QuantiBlot® Human DNA Quantitation Kit from Applied Biosystems. Chemiluminescent detection was performed using the ECL™ detection reagents (Amersham Biosciences, Buchinghamshire, England).

DNA Amplification

The DNA extracted by all methods from all volunteer solid fecal matters stored either at room temperature or at −20°C, fecal matters from volunteers deposited on tissue papers, the two types of fecal samples (solid lump and tissue papers) seized from the crime scene, the DNA extracted from all buccal swabs were amplified using the AmpFℓSTR® Profiler Plus™ PCR Amplification Kit (Applied Biosystems, Foster City, CA).

The DNA obtained using the QIAamp® DNA Stool Mini Kit and UltraClean™ Fecal DNA Isolation Kit extraction procedures from all of the male and female volunteer solid fecal samples stored either at room temperature, or at −20°C, were also amplified using the AmpFℓSTR® COfiler™ PCR Amplification Kit. Three of the male and two of the female volunteer samples, deposited on tissue papers and extracted by the two above described procedures, were amplified using the COfiler™ PCR Amplification Kit.

The DNA obtained from the three male volunteer solid fecal samples, either stored at room temperature or at −20°C, and extracted by the QIAamp® DNA Stool Mini Kit and UltraClean™ Fecal DNA Isolation Kit extraction procedures, were amplified using the AmpFℓSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems) and by the Y-PLEX™6 and Y-PLEX™5 Amplification Kits from ReliaGene Technologies, Inc. (New Orleans, LA). Fecal samples deposited on tissue papers from two male volunteers and extracted with the same extraction kits were also amplified using the Identifiler™ PCR Amplification Kit and by the Y-PLEX™6 and Y-PLEX™5 amplification kits.

None of the male or female volunteer samples extracted with conventional organic extraction procedure were amplified using the COfiler™ or the Identifiler™ PCR amplification kits. The DNA obtained from female samples stored under various conditions or deposited on tissue papers and extracted by the QIAamp® DNA Stool Mini Kit and UltraClean™ Fecal DNA Isolation Kit extraction procedures were not amplified using the Identifiler™ PCR Amplification Kit. None of the organically extracted male volunteer samples were subjected to amplification by Y-PLEX™6 and Y-PLEX™5 amplification kits.

The DNA extracted from the tissue papers soiled with fecal samples seized from the robbery crime scene and extracted by the organic extraction and the QIAamp® DNA Stool Mini Kit procedures were amplified using the COfiler™ and the Identifiler™ PCR amplification kits for detection of the autosomal STR profiles. This DNA sample was also amplified using Y-PLEX™6 and Y-PLEX™5 amplification kits for detection of the Y-STR profiles. The extracts obtained from the solid fecal matter from the robbery crime scene using all of the three extraction procedures were not amplified using the COfiler™, Identifiler™, or the Y-PLEX™6 and Y-PLEX™5 amplification kits.

The amount of DNA added to the PCR reactions was in the range of 0.5 to 1.5 ng in the amplification reaction used for the autosomal DNA amplification. For the Y-PLEX™6 and Y-PLEX™5 amplification kits, approximately 0.5 to 2.0-ng DNA template was used. Although a 50-μL reaction volume is recommended for the amplification of the AmpFℓSTR® Profiler Plus™ and AmpFℓSTR® COfiler™ amplification kits (9), all amplifications were carried out in 25-μL reaction volumes (8–11).

Amplification was performed as per each of the manufacturer's recommendations using the GeneAmp® 9700 Thermal Cycler (PE-Applied Biosystems, Foster City, CA).

Capillary Electrophoresis and Detection of STR Alleles

Amplified products were mixed with appropriate size standards and formamide as recommended by the manufacturers of the kits. Each sample was denatured for 3 min at 95°C and then snap-chilled on ice for 3 min. Amplified PCR products were analyzed by capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer instrument. Electrophoretic conditions and filter sets were determined as per the manufacturer's recommended protocols.

The data were analyzed by GeneScan® Analysis software versions 3.1.2, and Genotyper® software version 2.5.2 was used to make the autosomal allele calls. Y-STR alleles were typed by either Y6-Typer 310 version 4.0 or by Y5-Typer 310 version 1.0 in conjunction with the Genotyper® software. Peak amplitude threshold for GeneScan® analysis was set at 150 RFU for all systems. Alleles were assigned by comparison to appropriate allelic ladders.

Results and Discussion

STR analysis is becoming an integral part of forensic DNA analysis. The goal of this research was to detect STR alleles from stool samples. While human stool evidence is not commonly encountered in forensic cases, occasionally it may be the only evidence left by a perpetrator at a crime scene. The goal was to determine if concordant STR alleles could be obtained from stool samples using various commercially available kits.

This investigation was also carried out to determine if DNA could be obtained from human stool samples using commercially

available extraction kits followed by a concentration procedure. The objective was to establish if human stool samples could be used as a source for DNA for amplification with the Profiler Plus™, COfiler™, the Identifiler™ PCR amplification kits, and the Y-PLEX™6 and Y-PLEX™5 amplification kits. DNA extraction methods were compared using samples that were stored at room temperature or at −20°C for approximately eight weeks.

Tables 1*a*, 1*b*, and 1*c* illustrate the results of extraction, quantitation, and amplification of various samples from female volunteers. Using the QIAamp® DNA Stool Mini Kit and the UltraClean™ Fecal DNA Isolation Kit, complete autosomal STR profiles were detected from all female volunteer stool samples when the solid fecal matters were stored at room temperature or kept frozen for approximately eight weeks (Table 1*a* and 1*b*). Fecal matters deposited on tissue papers from two female volunteers gave complete autosomal STR profiles, whereas partial profiles were obtained from fecal matters deposited on tissue papers from two other females using the above two extraction procedures. No attempt was made to amplify any female DNA with the primers contained in the Identifiler™ Amplification Kit.

Partial autosomal profiles, using DNA obtained by organic extraction procedure in conjunction with the Rapid PCR Purification System, were obtained from two female stool samples stored at −20°C (Table 1*c*). None of the other female samples, whether stored at room temperature, or at −20°C, or fecal matters deposited on tissue papers, yielded any result using the organic extraction procedure.

Tables 2*a*, 2*b*, and 2*c* illustrate the results of extraction, quantitation, and amplification of various samples from the four male volunteers and the two types of fecal evidence samples collected from the crime scene. Using the QIAamp® DNA Stool Mini Kit

TABLE 1*a*—*Result obtained from fecal matters of females using QIAamp® DNA Stool Mini Kit.*

Donor and Amount of Stool Sample Extracted	Total Amount of DNA Obtained	Profiler Plus	COfiler
No. 1 Female Donor (RT): ~900 mg	4.0 ng	F	F
No. 1 Female Donor (−20°C): ~900 mg	2.0 ng	F	F
No. 1 Female Donor tissue paper (RT): ~500 mg	2.0 ng	F	F
No. 2 Female Donor (RT): ~900 mg	3.0 ng	F	F
No. 2 Female Donor (−20°C): ~900 mg	4.0 ng	F	F
No. 2 Female Donor tissue paper (RT): ~500 mg	0.3 ng	P	NT
No. 3 Female Donor (RT): ~900 mg	2.0 ng	F	F
No. 3 Female Donor (−20°C): ~900 mg	1.0 ng	F	F
No. 3 Female Donor tissue paper (RT): ~500 mg	0.2 ng	P	NT
No. 4 Female Donor (RT): ~900 mg	6.0 ng	F	F
No. 4 Female Donor (−20°C): ~900 mg	4.0 ng	F	F
No. 4 Female Donor tissue paper (RT): ~500 mg	2.0 ng	F	F

NR = no result; NT = not tested; F = full profile; P = partial profile (at least 2 loci detected); RT = room temperature; <0.15 ng = no visible band upon quantitation.

TABLE 1*b*—*Result obtained from fecal matters of females using UltraClean™ Fecal DNA Isolation Kit.*

Donor and Amount of Stool Sample Extracted	Total Amount of DNA Obtained	Profiler Plus	COfiler
No. 1 Female Donor (RT): ~900 mg	2.0 ng	F	F
No. 1 Female Donor (−20°C): ~900 mg	4.0 ng	F	F
No. 1 Female Donor tissue paper (RT): ~500 mg	3.0 ng	F	F
No. 2 Female Donor (RT): ~900 mg	3.0 ng	F	F
No. 2 Female Donor (−20°C): ~900 mg	4.0 ng	F	F
No. 2 Female Donor tissue paper (RT): ~500 mg	<0.15 ng	P	NT
No. 3 Female Donor (RT): ~900 mg	3.0 ng	F	F
No. 3 Female Donor (−20°C): ~900 mg	2.0 ng	F	F
No. 3 Female Donor tissue paper (RT): ~500 mg	0.3 ng	P	NT
No. 4 Female Donor (RT): ~900 mg	4.0 ng	F	F
No. 4 Female Donor (−20°C): ~900 mg	5.0 ng	F	F
No. 4 Female Donor tissue paper (RT): ~500 mg	3.0 ng	F	F

NT = Not tested.
F = Full profile.
P = Partial profile (at least 2 loci detected).
RT = Room temperature.
<0.15 ng = No visible band upon quantitation.

TABLE 1*c*—*Result obtained from fecal matters of females using Organic Extraction procedure.*

Donor and Amount of Stool Sample Extracted	Total Amount of DNA Obtained	Profiler Plus	COfiler
No. 1 Female Donor (RT): ~900 mg	<0.15 ng	NR	NT
No. 1 Female Donor (−20°C): ~900 mg	0.30 ng	P	NT
No. 1 Female Donor tissue paper (RT): ~500 mg	<0.15 ng	NR	NT
No. 2 Female Donor (RT): ~900 mg	<0.15 ng	NR	NT
No. 2 Female Donor (−20°C): ~900 mg	<0.15 ng	NR	NT
No. 2 Female Donor tissue paper (RT): ~500 mg	<0.15 ng	NR	NT
No. 3 Female Donor (RT): ~900 mg	<0.15 ng	NR	NT
No. 3 Female Donor (−20°C): ~900 mg	<0.15 ng	P	NT
No. 3 Female Donor tissue paper (RT): ~500 mg	<0.15 ng	NR	NT
No. 4 Female Donor (RT): ~900 mg	<0.15 ng	NR	NT
No. 4 Female Donor (−20°C): ~900 mg	<0.15 ng	NR	NT
No. 4 Female Donor tissue paper (RT): ~500 mg	<0.15 ng	NR	NT

NR = No result; N = not tested; F = full profile; P = partial profile (at least 2 loci detected); RT = room temperature; <0.15 ng = no visible band upon quantitation.

TABLE 2*a*—*Result obtained from fecal matters of males using QIAamp® DNA Stool Mini Kit procedure*.

Donors and Amount of Stool Sample Extracted	Total Amount of DNA Obtained	Profiler Plus	COfiler	Identifiler	Y-LEX™5	Y-PLEX™6
No. 1 Male Donor (RT): ~900 mg	3.0 ng	F	F	F	F	F
No. 1 Male Donor (−20°C): ~900 mg	5.0 ng	F	F	F	F	F
No. 2 Male Donor (RT): ~900 mg	2.0 ng	F	F	NT	NT	NT
No. 2 Male Donor (−20°C): ~900 mg	2.0 ng	F	F	NT	NT	NT
No. 2 Male Donor tissue paper (RT): ~500 mg	1.5 ng	F	F	NT	NT	NT
No. 3 Male Donor (RT): ~900 mg	4.0 ng	F	F	F	F	F
No. 3 Male Donor (−20°C): ~900 mg	3.0 ng	F	F	F	F	F
No. 3 Male Donor No. 3 tissue paper (RT): ~500 mg	4.0 ng	F	F	F	F	F
No. 4 Male Donor (RT): ~900 mg	3.5 ng	F	F	F	F	F
No. 4 Male Donor (−20°C): ~900 mg	4.0 ng	F	F	F	F	F
No. 4 Male Donor tissue paper (RT): ~500 mg	3.5 ng	F	F	F	F	F
Fecal evidence: (−20°C): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
Fecal evidence on tissue paper (−20°C): ~500 mg	3.0 ng	F	F	F	F	F

NR = no result; NT = not tested; F = full profile; P = partial profile (at least 2 loci detected); RT = room temperature; <0.15 ng = no visible band upon quantitation.

TABLE 2*b*—*Result obtained from fecal matters of males using UltraClean™ Fecal DNA Isolation Kit.*

Donor and Amount of Stool Sample Extracted	Total Amount of DNA Obtained	Profiler Plus	Cofiler	Identifiler	Y-PLEX™5	Y-PLEX™6
No. 1 Male Donor (RT): ~900 mg	5.0 ng	F	F	F	F	F
No. 1 Male Donor (−20°C): ~900 mg	6.0 ng	F	F	F	F	F
No. 2 Male Donor (RT): ~900 mg	2.0 ng	F	F	NT	NT	NT
No. 2 Male Donor (−20°C): ~900 mg	1.0 ng	F	F	NT	NT	NT
No. 2 Male Donor tissue paper (RT): ~500 mg	1.0 ng	F	F	NT	NT	NT
No. 3 Male Donor (RT): ~900 mg	4.0 ng	F	F	F	F	F
No. 3 Male Donor (−20°C): ~900 mg	4.0 ng	F	F	F	F	F
No. 3 Male Donor tissue paper (RT): ~500 mg	3.5 ng	F	F	F	F	F
No. 4 Male Donor (RT): ~900 mg	3.5 ng	F	F	F	F	F
No. 4 Male Donor (−20°C): ~900 mg	4.0 ng	F	F	F	F	F
No. 4 Male Donor tissue paper (RT): ~500 mg	3.0 ng	F	F	F	F	F
Fecal evidence: (−20°C): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
Fecal evidence on tissue paper (−20°C)	NT	NT	NT	NT	NT	NT

NR = No result; NT = Not tested; F = Full profile; P = Partial profile (at least 2 loci detected); RT = Room temperature; <0.15 ng = No visible band upon quantitation.

TABLE 2*c*—*Result obtained from fecal matters of males using Organic Extraction procedure*.

Donor and Amount of Stool Sample Extracted	Total Amount of DNA Obtained	Profiler Plus	Cofiler	Identifiler	Y-PLEX™5	Y-PLEX™6
No. 1 Male Donor (RT): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
No. 1 Male Donor (−20°C): ~900 mg	0.3 ng	P	NT	NT	NT	NT
No. 2 Male Donor (RT): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
No. 2 Male Donor (−20°C): ~900 mg	<0.15 ng	P	NT	NT	NT	NT
No. 2 Male Donor tissue paper (RT): ~500 mg	<0.15 ng	NR	NT	NT	NT	NT
No. 3 Male Donor (RT): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
No. 3 Male Donor (−20°C): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
No. 3 Male Donor tissue paper (RT): ~500 mg	<0.15 ng	NR	NT	NT	NT	NT
No. 4 Male Donor (RT): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
No. 4 Male Donor (−20°C): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
No. 4 Male Donor tissue paper (RT): ~500 mg	<0.15 ng	NR	NT	NT	NT	NT
Fecal evidence: (−20°C): ~900 mg	<0.15 ng	NR	NT	NT	NT	NT
Fecal evidence on tissue paper (−20°C): ~ 500 mg	3.0 ng	F	F	F	F	F

NR = No result; NT = Not tested; F = Full profile; P = Partial profile (at least 2 loci detected); RT = Room temperature; <0.15 ng = No visible band upon quantitation.

and the UltraClean™ Fecal DNA Isolation Kit, complete autosomal STR profiles were detected from three male volunteer stool samples when the solid fecal matters were stored at room temperature or kept frozen for approximately eight weeks. Fecal matters deposited on tissue papers from three male volunteers gave complete autosomal STR profiles using the above two extraction procedures (Tables 2*a* and 2*b*) when amplified with the Profiler Plus™ and the COfiler™ Amplification Kits. The DNA, extracted by the QIAamp® DNA Stool Mini Kit and the UltraClean™ Fecal DNA Isolation Kit from two of these tissue paper samples, were successfully amplified using the Identifiler™ PCR Amplification Kit.

As the above two tables indicate, complete Y-STR profiles were detected from the three volunteer male solid stool samples stored at room temperature and at −20°C using the two above-described extraction procedures. Complete Y-STR profiles were also detected from two male stool samples deposited on tissue papers.

Using conventional organic extraction and the Rapid PCR Purification System, partial autosomal profiles from two volunteer male samples were obtained when using frozen samples (Table 2*c*). Using this extraction procedure, no autosomal profiles were detected from any other sample stored at room temperature or at −20°C or from samples deposited on tissue papers. No attempt was made to amplify DNA from any male volunteer sample extracted with the organic extraction procedure by the Identifiler™ or the Y-PLEX™6 and Y-PLEX™5 amplification kits.

The solid stool evidence sample obtained at the robbery crime scene and extracted using the three extraction procedures described above yielded no DNA profile. The soiled tissue paper evidence sample seized at the same crime scene is presumed to have been used to clean the anal area following defecation. This evidence sample upon extraction by the organic extraction and the QIAamp® DNA Stool Mini Kit yielded complete profiles of all autosomal and Y-STR loci. Figure 1 illustrates the results of DNA extracted by organic extraction and amplified with the Identifiler™ Amplification Kit. Alleles at the 13 loci common to Identifiler™, Profiler Plus™, and COfiler™ gave concordant results and were completely consistent with the results obtained from the suspect's buccal swab (data not shown).

Figure 2 indicates the Genotyper® profile for the five Y-STR loci detected from the fecal matters deposited on the tissue paper obtained from the crime scene and extracted with the QIAamp® DNA Stool Mini Kit. Extracted DNA was amplified with the Y-PLEX™5 Primers.

The Genotyper® profile for the six Y-STR loci detected from the stool sample of a male volunteer is depicted in Fig. 3. A sample was stored at room temperature for approximately eight weeks and extraction was performed using the UltraClean™ Fecal DNA Isolation Kit. Extracted DNA was amplified with the Y-PLEX™6 Primers. Y-PLEX™6 and Y-PLEX™5 amplification kits together supply primers for amplification at ten loci with one overlapping locus, DYS389II. Figure 2 and Fig. 3 indicate two different male profiles. Both of the donors yielded concordant results regarding the overlapping locus DYS389II using the two kits (data not shown).

Y-STR typing was not attempted when fecal matters on tissue papers were organically extracted from the male volunteers, since the amount of DNA obtained from these samples was low.

The amount of DNA obtained from fecal samples varied. The DNA yield ranged from 0.5 to 6 ng using the QIAamp® DNA Stool Mini Kit and the UltraClean™ Fecal DNA Isolation Kit. Attempts were made to scrape only the outer edges of the solid stools since the epithelial cells are expected to be more abundant in this surface

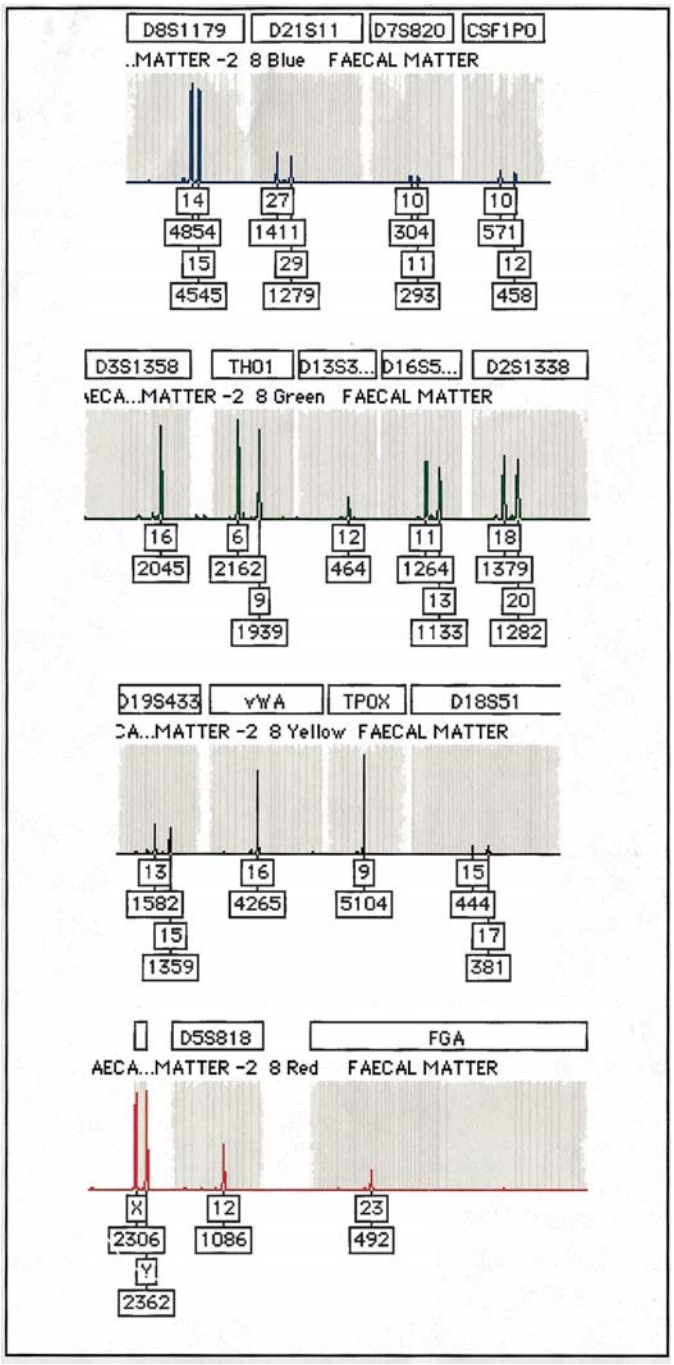

FIG. 1—*Genotyper® Profile detected from fecal matter deposited on tissue paper seized at a crime scene. Sample was extracted using organic extraction procedure and amplified using AmpF€STR® Identifiler™ PCR Amplification Kit. The 16 loci depicted above are the following: D8S1179, D21S11, D7S820, CSF1PO (blue), D3S1358, TH01, D13S317, D16S539, D2S1338 (green), D19S433, vWA, TPOX, D18S51 (yellow), D5S818, FGA, and amelogenin (red).*

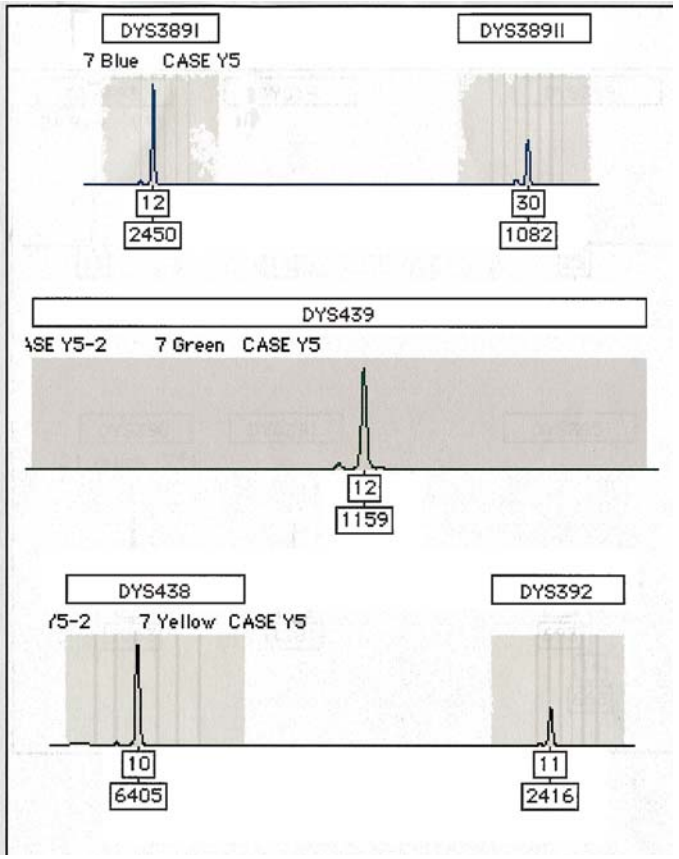

FIG. 2—*Genotyper® Profile detected from fecal matter deposited on tissue paper obtained at the robbery crime scene. Sample was extracted by QIAamp® DNA Stool Mini Kit procedure and amplified using the Y-PLEX™5 Amplification Kit. The five loci depicted above are the following: DYS389I, DYS389II (blue), DYS439 (green), DYS438, and DYS392 (yellow).*

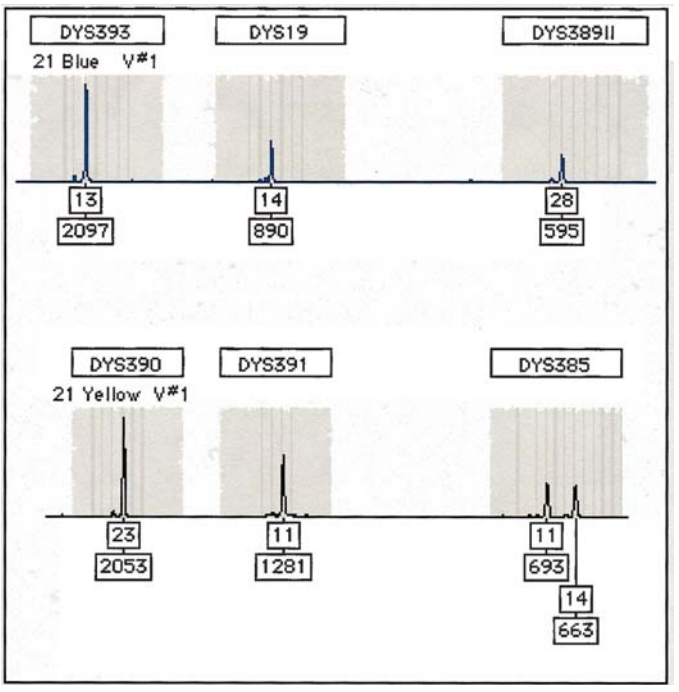

FIG. 3—*Genotyper® Profile detected from fecal matter obtained from a male volunteer. Sample was extracted after storage at room temperature for approximately eight weeks by the UltraClean™ Fecal DNA Isolation Kit procedure and amplified using Y-PLEX™6 Amplification Kit. The six loci depicted above are the following: DYS393, DYS19, DYS389II (blue), DYS390, DYS391, and DYS385 (yellow).*

area. No difference in the quantity or quality of DNA or subsequent ability to amplify was observed using either the QIAamp® DNA Stool Mini Kit and the UltraClean™ Fecal DNA Isolation Kit. The organic extraction procedure appeared to yield approximately 0.3 to 1.0 ng of DNA from 900 to 500 ng of fecal matter and soiled tissue paper, respectively. This extraction method appears to be the least effective. However, the tissue paper containing fecal matter seized at the crime scene was extracted successfully.

The results of the current research indicate that it is possible to obtain human DNA from fecal materials using both the QIAamp® Mini Stool Kit and the UltraClean™ Fecal DNA Isolation Kit. Using the AmpFℓSTR® Profiler Plus™, AmpFℓSTR® COfiler™, and the AmpFℓSTR® Identifiler™ PCR amplification kits, it was possible to detect autosomal STR alleles from stool samples stored either at −20°C or at room temperature. For situations where the only available evidence is fecal matter, the results indicate that successful profiles may be obtained when appropriate DNA extraction methods are employed.

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References

1. Al-Soud WA, Rådström P. Capacity of nine thermostable DNA polymerases to mediate DNA amplification in the presence of PCR-inhibiting samples. Appl Env Microbiol 1998;64:3740–53.
2. Monteiro L, Bonnemaison D, Vekris A, Petry KG, Bonnet J, et al. Complex polysaccharides as PCR inhibitors in feces. J Clin Microbiol 1997: 995–8.
3. Deuter R, Peetsch S, Hertel S, Muller O. A method for preparation of fecal DNA suitable for PCR. Nucleic Acids Res 1995;23(18):3800–1.
4. Comey CT, Koons BW, Presley KW, Smerick JB, Sobieralski CA, Stanley DM, et al. DNA extraction strategies for amplified fragment length polymorphism analysis. J Forensic Sci 1994;39:1254–69.
5. Gangitano DA, Garófalo MG, Juvenal GJ, Budowle B, Padula RA. Typing of the locus DYS19 from DNA derived from fingernail clippings using PCR Concert™ rapid purification system. J Forensic Sci 2002; 47(1):175–7.
6. QIAGEN. QIAamp DNA stool mini kit handbook 2001:8.
7. MO BIO Laboratories. Fecal DNA isolation kit handbook 2001:6.
8. Applied Biosystems. AmpFℓSTR® Identifiler™ PCR Amplification Kit. User's manual, 2001.
9. Applied Biosystems AmpFℓSTR® Profiler Plus™ and COfiler™ PCR Amplification Kit. User's manual, 1998.
10. ReliaGene Technologies. Y-PLEX™5 Amplification Kit. Instruction manual. 2002:1.
11. ReliaGene Technologies. Y-PLEX™6 Amplification Kit. Instruction manual. 2002:5.

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