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TECHNICAL NOTE

CRIMINALISTICS; ANTHROPOLOGY

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Comparison of Two Methods for Isolating DNA from Human Skeletal Remains for STR Analysis

ABSTRACT: The quality and efficiency of a standard organic DNA isolation method and a silica-based method using the QIAGEN Blood Maxi Kit were compared to obtain human DNA and short tandem repeats (STRs) profiles from 39 exhumed bone samples for paternity testing. DNA samples were quantified by real-time PCR, and STR profiles were obtained using the AmpF*l*STR® Identifiler® PCR amplification kit. Overall, the silica-based method recovered less DNA ranging from 0 to 147.7 ng/g (average 7.57 ng/g, median = 1.3 ng/g) than did the organic method ranging from 0 to 605 ng/g (average 44.27 ng/g, median = 5.8 ng/g). Complete profiles (16/16 loci tested) were obtained from 37/39 samples (95%) using the organic method and from 9/39 samples (23%) with the silica-based method. Compared with a standard organic DNA isolation method, our results indicate that the published silica-based method does not improve neither the quality nor the quantity of DNA for STR profiling.

KEYWORDS: forensic science, DNA typing, DNA isolation, human skeletal remains, real-time PCR, STR, Plexor HY, FGA, TPOX, D8S1179, vWA, D18S51, D21S11, TH01, D3S1358, D19S433, D2S1338, CSF1PO, D16S539, D7S820, D13S317, D5S818, amelogenin

Human skeletal remains have been used as a source of DNA for human identification in natural mass disasters (1–3), terrorist attacks (4,5), airplane crashes, cases of missing persons, wars and conflicts (6,7), mass graves (8), and paternity cases among others. These remains represent one of the most challenging types of samples for obtaining DNA for processes of human identification. Many factors including heat, humidity, and the presence of PCR inhibitors at the inhumation site (9) influence both quality and quantity of DNA for casework analysis (10).

Many protocols have been described for isolating DNA from human remains (11–16). Some of them are time-consuming, use toxic reagents, and require multiple steps to obtain DNA suitable for short tandem repeats (STR) analysis. Many laboratories around the world, including ours, use a phenol–chloroform-based DNA isolation method to obtain STR genetic profiles from human bone samples. Using this method for more than 12 years, we have obtained a success rate between 95 and 98% for the generation of complete STR (15 loci) and amelogenin profiles, using either PowerPlex® 16 or AmpF*l*STR® Identifiler® PCR amplification kit from 4 g of bone powder. Recently, a silica-based method was published by the International Commission of Missing Persons (ICMP). It improves turnaround time and reduces the use of toxic reagents needed to obtain STR profiles (13,17). This kind of methodology could be extremely helpful in conflict ridden countries where DNA-based methods for identification are needed because of the large number of victims, and in many cases, the lack of dental and fingerprint records or other means of identification.

For the last 45 years, Colombia has been involved in an armed conflict. Guerrilla forces were formed (The *Fuerzas Armadas Revolucionarias de Colombia-FARC,* the *Ejército de Liberación Nacional-ELN,* and the *Movimiento 19 de Abril-M19*, among others) to fight against regular military forces (18–20). Paramilitary forces were also created during the last 30 years. The number of victims of this conflict is still unclear. Estimates ranged from 3000 to 8000 depending on the source (21). Recent official data indicate that there are 32,862 missing persons because of the internal conflict (22). After the Peace and Justice Law of 2005 (Ley de Justicia y Paz o Ley 975 de 2005) was signed in Colombia, most of the paramilitary forces and some guerrilla members surrendered to the government and began confessing their crimes. As of June 30, 2010, the Fiscalía General de la Nación (Colombian Department of Justice) had reported that 3299 human remains had been recovered from 2719 mass graves (22). These recovered bodies represent a small portion of the total number of bodies still to be exhumed as there are many locations with difficult access because of continued fighting (23), more mass graves are yet to be discovered, and more locations may be disclosed by the paramilitary and guerrilla members who have surrendered.

This study compares a standard organic-based DNA isolation method to the silica-based method recommended by the ICMP to generate STR profiles from human skeletal remains to test for paternity. The comparison was undertaken to determine whether a faster and less toxic method could be introduced to different laboratories in Colombia to obtain high-quality STR profiles from skeletal human remains to be used not only for legal assessment of

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paternity, but also for testing biological relationships for human identification purposes for future cases.

Materials and Methods

Bone Samples

Thirty-nine bone samples from exhumed human skeletal remains were used for identification related to legal assessment of paternity. The samples included 14 tibias and 25 femurs: 38 samples were men and one was a female. The human remains had been buried for an average of 37 months (range 6–118 months). Proper legal consent was obtained for all cases. Samples came from different regions of the country ranging from warm and humid to cool and dry climates.

Laboratory

An ISO/IEC 17025:2005 laboratory facility dedicated for human skeletal remain was used to process all samples. Manual cleaning, grinding, and DNA extraction procedures were performed in separate spaces to avoid contamination with DNA from high-yield DNA samples. Appropriate hair nets, facemasks, lab coats, and gloves were used throughout the procedures. The PCR and real-time PCR setups were performed in a separate laboratory facility.

Cleaning and Grinding of Bone Samples

Each skeletal remain surface was cleaned. Soft tissue, dirt, and 2 mm of the external and internal bone surfaces were removed by using a high-speed rotary sanding tool (Dremel 300-1/25; Racine, WI). The bones were fragmented by using a properly sterilized rubber mallet. Bone fragments were immersed in distilled water for 30 sec, then 5% commercial bleach for 30 sec followed by 96% ethanol for 30 sec. Then bone fragments were dried at 50°C for 2 h or at room temperature overnight.

Cleaned bone fragments were pulverized under sterile conditions with a SPEX SamplePrep 6770 Freezer/Mill cryogenic impact grinder (Spex, Metuchen, NJ). Samples were pulverized under liquid nitrogen with three cycles alternating 1-min impacting and 1-min holding. The resulting bone powder was then divided into two tubes. Two grams were placed in each tube for the standard organic isolation method, and between 2.4 and 6.0 g of bone powder was placed in each tube for the silica-based method. Weighing of powdered bone samples was performed in a pre-PCR weighing room under sterile conditions using a Mettler BB600 balance (Mettler-Toledo, Columbus, OH). Testing for reagent blank samples was begun at this point for both DNA extraction methods.

Silica-Based DNA Extraction

The protocol was based on QIAGEN's Blood Maxi Kit (Hilden, Germany) with modifications as described by Davoren et al. (13). The bone powder (2.4–6.0 g/tube) was incubated with 15 mL of ATL buffer containing 300 μL of 1 M DTT (Promega Corporation, Madison, WI), 200 μL of proteinase K at 20 mg/mL (QIAGEN) for 18 h at 56°C in a shaking water bath, along with a reagent blank sample. A supplemental digestion was carried out by adding 14 mL of AL buffer, mixing for 30 sec by inversion and incubating at 70°C for 1 h in a shaking water bath. The remaining bone material was removed by centrifugation at $1000 \times g$ for 5 min in a Clay Adams Dynac centrifuge (BD, Franklin Lakes, NJ). Then supernatant from both extracts was transferred to separate 50-mL tubes. Twenty-two milliliters of 96% ethanol was added to each tube and mixed by inversion. The DNA was bound to QIAGEN Blood Maxi columns by adding 15 mL of the extraction mix and centrifuging at $2000 \times g$ for 3 min. Flow-through liquid was discarded. This process of adding 15 mL of extraction mix, centrifuging for 3 min at $2000 \times g$, and discarding the flow-through liquid was repeated until all the extraction mix was bound to the column. The columns were then washed with 10 mL of prepared AW1 buffer, followed by centrifugation at $2000 \times g$ for 3 min. A second wash was performed with 10 mL of AW2 buffer. The remaining AW2 buffer was removed by centrifugation for 10 min at $2000 \times g$. The column was transferred to a new 15-mL tube, and the DNA was eluted by the addition of 3 mL of AE buffer preheated to 72°C. The column was then incubated at room temperature for 5 min and spun down for 3 min at $2000 \times g$. A second elution was performed exactly as the first one. Six milliliters of eluted DNA from each tube was combined into a single 15-mL tube. The DNA was concentrated using a single 15-mL Centriplus YM-100 column (Millipore, Billerica, MA) and spun down for 10 min at $2000 \times g$. The retentate was washed three times with the addition of 4 mL of molecular grade water each time followed by centrifugation at $2000 \times g$ for 10 min. The Centriplus YM-100 column membrane was washed with 110 μL of water to recover the DNA, and the concentrated DNA was transferred to 1.5-mL tubes. No additional concentration steps were performed.

Organic Extraction

For each sample, a total of 4 g of bone powder was divided into two 15-mL tubes. The samples and a reagent blank sample were decalcified three times with EDTA 0.5 M pH 8.0 (Promega Corporation) for 16 h in a rotary shaker with agitation at room temperature. The samples were centrifuged at $2000 \times g$ for 10 min. The supernatant was discarded, and the remaining bone was resuspended in 13 mL of distilled water, mixed for 10 sec, and spun down at $2000 \times g$ for 10 min. The supernatant was discarded, and the distilled water wash was repeated two more times. Then, 6 mL of lysis buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 50 mM EDTA, pH 8.0, 0.5% SDS; Promega Corporation) containing 200 μL of proteinase K (20 mg/mL) (Promega Corporation) was added and incubated at 56°C for 16–24 h. After centrifugation, the supernatant was transferred to a 15-mL tube containing 6 mL of phenol–chloroform–isoamyl alcohol (25:24:1). The samples were mixed until a homogeneous solution was obtained. The aqueous phase was separated by centrifugation at $2000 \times g$ for 20 min and transferred to a new tube. This extraction step was repeated two more times, followed by another extraction, this time with 6 mL of chloroform–isoamyl alcohol (24:1). Then, the aqueous phase from both extracts from each sample was combined into one tube, concentrated using a 15-mL Centriplus YM-100 column (Millipore, Billerica, MA), and spun down for 10 min at $2000 \times g$. The retentate was then washed with the addition of 4 mL of molecular grade water followed by centrifugation at $2000 \times g$ for 10 min. This was repeated three times. The Centriplus YM-100 column membrane was washed with 110 μL of water to recover DNA, and then the concentrated DNA was transferred to 1.5-mL tubes.

DNA Quantification

DNA samples were quantified by real-time PCR with the Plexor HY Kit (Promega Corporation) in an MJ Research®

Chromo4 thermal cycler (MJ Research, Watertown, MA) and analyzed with the Plexor Analysis Software—v1.5.6.2 (Promega Corporation) according to the manufacturer's recommendations (with the exception that samples were not quantified in duplicate). The Plexor HY system allows simultaneous quantitation of autosomal and Y-chromosome DNA in each sample. The internal PCR control (IPC) included in each sample was used to detect PCR inhibitors in the DNA extracts. PCR inhibitors retard the onset of exponential amplification in the PCR, which can be detected as an increase in the cycle threshold (Ct) value for IPC. Negative controls and reagent blanks were included in every step of the study. A dilution curve using known concentrations of standards expressed as ng/μL was used to normalize and quantify the amount of autosomal and Y-chromosome DNA present in each sample. The total amount of autosomal DNA was then calculated per gram of bone used based on the total volume of DNA sample recovered, Ct values for autosomal DNA, and the total mass of bone used for DNA isolation (Table 1).

Amplification and Analysis

DNA samples obtained from both methods were used for STR analysis using the AmpF*l*STR® Identifiler® PCR amplification kit following recommendations from the manufacturer (Applied Biosystems, Foster City, CA) in a PTC 100 (MJ Research) thermal cycler. The DNA amount used in 10 μL of sample for the PCR is listed in Table 1. In a few instances, the DNA was diluted to obtain <10 ng of DNA in the 10 μL used. On average, 1.9–2.0 ng of DNA/10 μL was used for PCR amplification from samples isolated with both methods (Table 1). PCR amplification consisted of a first cycle at 95°C for 11 min, 32 cycles at 94°C for 1 min, 59°C for 1 min and 72°C for 1 min (ramping time 1°C/sec), and 1 cycle at 60°C for 60 min. STR fragments were analyzed on an Applied Biosystems 3130xl Genetic Analyzer using POP4 polymer with injection voltage of 3.0 kV, 10 sec of injection time, and 15-kV run voltage. Positive controls (provided by the manufacturer), negative controls, and reagent blank controls were analyzed in each run.

TABLE 1—*DNA yields, Ct values, and AmpF*l*STR® Identifiler® profile results for skeletal human remains tested in this study.*

	Silica-Based DNA Extraction Method								Organic DNA Extraction Method							
Code	Type	Bone (g)	IPC (Ct)	Autosomal (Ct)	Concentration (ng/μL)	DNA Yield (ng/g)	ng DNA/ PCR	# Loci	Type	Bone (g)	IPC (Ct)	Autosomal (Ct)	Concentration (ng/μL)	DNA Yield (ng/g)	ng DNA/ PCR	# Loci
95096	Femur	6.0	15.0	20.3	0.098	1.797	0.980	14	Femur	4	16.6	18.4	0.390	10.725	1.950	16
97351	Femur	7.5	15.3	18.8	0.300	4.400	3.000	14	Femur	4	14.4	19.2	0.220	6.050	1.100	16
97466	Femur	8.0	16.1	21.8	0.035	0.481	0.350	5	Femur	4	12.5	12.7	22.000	605.000	8.800	16
98169*	Femur	7.0	24.7	17.8	9.400	147.714	9.400	10	Femur	4	23.9	16.7	21.000	577.500	8.400	16
98215*	Femur	6.5	23.7	22.25	0.435	7.362	4.350	11	Femur	4	21.1	31.8	0.001	0.022	0.004	16
100732	Femur	7.0	16.8	23.9	0.008	0.119	0.076	3	Femur	4	16.2	20.1	0.120	3.300	0.600	16
100927	Femur	7.0	16.2	21.0	0.060	0.943	0.600	11	Femur	4	15.4	15.0	4.200	115.500	4.200	16
101053*	Femur	7.5	22.5	24.9	0.449	6.585	4.490	0	Femur	4	22.5	26.0	0.041	1.128	0.205	16
101099*	Femur	6.5	22.9	25.5	0.050	0.838	0.495	10	Femur	4	N/A	N/A	0.000	0.000	0.000	16
103538*	Femur	4.8	20.5	30.05	0.003	0.057	0.025	0	Femur	4	26.0	35.3	0.009	0.234	0.043	16
105216	Femur	7.5	23.4	27.1	0.260	3.813	2.600	13	Femur	4	21.3	27.4	0.210	5.775	1.050	16
105340	Femur	8.5	21.4	25.9	0.540	6.988	5.400	16	Femur	4	23.4	27.2	0.250	6.875	1.250	16
105682	Femur	5.5	21.9	30.1	0.040	0.800	0.400	16	Femur	4	21.4	25.2	0.860	23.650	4.300	16
105733	Femur	6.0	21.8	29.2	0.071	1.302	0.710	15	Femur	4	25.8	22.9	3.400	93.500	3.400	16
106861	Femur	5.0	23.0	33.5	0.005	0.110	0.050	0	Femur	4	25.6	25.9	0.540	14.850	2.700	16
107256	Femur	6.5	21.6	29.2	0.069	1.168	0.690	7	Femur	4	22.1	24.1	1.700	46.750	8.500	16
107467	Femur	7.5	21.9	36.2	0.001	0.014	0.009	1	Femur	4	24.2	27.1	0.260	7.150	1.300	16
108076	Femur	7.0	23.1	35.0	0.002	0.031	0.020	1	Femur	4	22.8	26.2	0.460	12.650	2.300	16
109066	Femur	8.0	23.5	26.4	0.390	5.363	3.900	16	Femur	4	23.9	26.0	0.520	14.300	2.600	16
109408	Femur	7.5	23.4	30.5	0.031	0.455	0.310	11	Femur	4	21.1	34.0	0.004	0.102	0.074	16
109710	Femur	7.5	22.0	25.4	0.720	10.560	7.200	15	Femur	4	24.9	25.3	0.790	21.725	3.950	16
110176	Femur	9.0	22.5	30.8	0.027	0.330	0.270	9	Femur	4	20.2	29.3	0.066	1.815	0.330	16
110179	Femur	12.0	23.1	23.8	2.000	18.333	2.000	10	Femur	4	22.6	23.1	3.000	82.500	3.000	16
110562	Femur	6.0	21.4	28.6	0.100	1.833	1.000	6	Femur	4	24.7	28.2	0.130	3.575	0.650	16
110846	Femur	6.5	22.6	29.8	0.048	0.812	0.480	12	Femur	4	23.5	27.5	0.200	5.500	1.000	16
90565*	Tibia	7.5	22.3	36.1	0.001	0.014	0.010	0	Tibia	4	N/A	N/A	0.000	0.000	0.000	0
98214	Tibia	5.5	15.3	19.5	0.180	3.600	1.800	16	Tibia	4	18.2	19.8	0.140	3.850	0.700	16
98277	Tibia	5.0	16.3	17.5	0.710	15.620	7.100	16	Tibia	4	14.7	19.8	0.140	3.850	0.700	16
100969*	Tibia	7.0	22.3	27.05	0.019	0.299	0.190	6	Tibia	4	29.3	27.0	0.021	0.564	0.103	16
101670	Tibia	7.0	15.3	18.1	0.470	7.386	4.700	16	Tibia	4	16.2	19.1	0.240	6.600	1.200	16
101812	Tibia	8.0	15.5	23.0	0.014	0.193	0.140	16	Tibia	4	14.6	18.0	0.520	14.300	2.600	16
101929*	Tibia	4.8	24.1	25.4	0.075	1.719	0.750	12	Tibia	4	22.4	27.8	0.095	2.619	0.476	16
103468	Tibia	7.5	15.4	19.0	0.250	3.667	2.500	16	Tibia	4	15.2	19.0	0.250	6.875	1.250	16
104001*	Tibia	9.5	23.1	29.6	0.056	0.648	0.560	0	Tibia	4	N/A	N/A	0.000	0.000	0.000	0
105090*	Tibia	8.0	22.3	26.6	0.360	4.950	3.600	16	Tibia	4	N/A	N/A	0.000	0.000	0.000	16
105412	Tibia	6.0	22.8	28.2	0.130	2.383	1.300	7	Tibia	4	24.2	28.9	0.084	2.310	0.420	16
107114*	Tibia	5.0	21.4	N/A	0.000	0.000	0.000	0	Tibia	4	N/A	N/A	0.000	0.000	0.000	16
108684	Tibia	7.0	21.6	23.8	2.000	31.429	4.000	12	Tibia	4	21.6	25.3	0.790	21.725	3.950	16
108741*	Tibia	7.0	20.5	29.1	0.075	1.179	0.750	12	Tibia	4	25.7	28.0	0.150	4.125	0.750	16
	Average	7.0	20.7		0.500	7.572	1.954		Average	4	21.1		1.610	44.282	1.894	

N/A, no amplification results with Plexor HY; ng DNA/PCR, amount of DNA used in 10-μL reaction for AmpF*l*STR® Identifiler® amplification; # Loci, number of AmpF*l*STR® Identifiler® loci with results.

*Samples quantified with Plexor HY in separate occasions to confirm results.

The final data were analyzed using GeneMapper ID v3.2 software (Applied Biosystems) to assign allele calls based on allelic ladders provided by the manufacturers. Samples were classified as those with complete profiles (15 STR loci alleles plus amelogenin), those with high partial profiles (8–15 loci), and those with low partial profiles (1–8 loci) with allele peak heights of at least 100 RFU. No amplifications were performed for Y-chromosome STR.

Results

DNA amounts and AmpF*l*STR® Identifiler® profile results obtained from the two methods used are shown in Table 1. The presence of PCR inhibitors was assessed on the basis of the Ct for the IPC in each sample. No significant differences were observed between the two methods used for DNA isolation. The average IPC Ct was 20.7 for the silica method and 21.1 for the organic method. Five samples isolated with the organic extraction method (90565, 104001, 105090, 101099, and 107114; four tibias and one femur) failed to amplify (IPC, autosomal, and Y chromosome) with the Plexor HY system despite the fact that a common master mix containing IPC was used for all samples (Table 1). Quantitation of these five samples along with other selected samples (labeled as * in Table 1) was repeated to verify results. Ct values similar to those obtained in the first quantification were found (data not shown).

The DNA yield results were normalized to determine the amount of DNA per gram of bone for each sample based on the DNA concentration (Ct values for autosomal DNA), the final volume of recovered DNA, and the mass of bone used for DNA isolation. With the silica-based method, an average of 7.57 ng/g of bone (range 0–147.71 ng/g) was obtained, while an average of 44.3 ng/g of bone (range 0–605 ng/g) was obtained with the organic extraction method. It should be noted that a smaller amount of DNA per gram of bone (ng/g) was obtained with the silica-based method than with the organic extraction method, despite the fact that in the silica-based method a higher amount of bone powder was used as starting material for DNA isolation.

Profile Results

Out of 39 samples tested, complete genetic profiles (15 STR loci plus amelogenin) were obtained from 37 (95%) DNA samples isolated using the organic extraction protocol, while only 9 of 39 DNA samples (23%) isolated with the silica-based method gave complete profiles that were identical to those obtained with the organic extraction protocol (Table 2). The STR profiles obtained in the 37 samples isolated with the organic extraction method were unique STR profiles. Reagent blanks and negative controls showed no signals. The bone samples were analyzed for postmortem paternity cases, and 78% of samples (29/37 samples) gave a "no exclusion" result with paternity indexes above 10,000 (as required by Colombian law). Altogether, these results ruled out any contamination from another sample. Neither isolation method was able to provide STR profiles from two DNA samples (ID# 90565 and 104001). These samples also failed to amplify with the Plexor HY system. Three samples isolated with the organic extraction method amplified a full AmpF*l*STR® Identifiler® profile (ID# 105090, 107114, and 101099) with apparently no DNA based on the PCR quantification. The quantification results were repeated for these samples with identical results. For the same samples isolated with the silica-based method, sample 107114 showed no DNA based on the autosomal Ct value and failed to amplify any of the AmpF*l*STR® Identifiler® loci tested. Sample 105090 yielded a complete AmpF*l*STR® Identifiler® profile, while only a partial profile was obtained for sample 101099. The overall results for the silica-based isolation samples showed high partial profiles (8–15 loci) in 16/39 samples (41%), low partial profiles (1–7 loci) in 8/39 samples (21%), and no profiles in 6/39 samples (15%). There was concordance for the STR results among samples that showed complete AmpF*l*STR® Identifiler® profiles with both methods. High and low partial profiles were concordant in the majority of samples. However, peak imbalances, allele drop out, and stutter artifacts not seen in the organic isolation samples were detected in the silica-based isolation samples (Fig. 1).

There was no clear correlation between the amount of starting bone material (gram), the yield of DNA, and the STR profile obtained with either method. For instance, samples 98277, 101670, 98215, 101053, and 108684 yielded larger amounts of DNA (ng/g of bone) with the silica-based method than with the organic extraction method, yet complete AmpF*l*STR® Identifiler® profiles were not obtained in all of them (98215, 101053, and 108684).

TABLE 2—*AmpFlSTR® Identifiler® profile results of DNA extracted from skeletal human remains using silica-based or organic extraction method.*

	Silica Based		Organic Extraction	
AmpF*l*STR® Identifiler® Profiles	No.	%	No.	%
Complete (16 loci)	9	23	37	95
High partial (8–15 loci)	16	41	0	0
Low partial (1–7 loci)	8	21	0	0
No results (0 loci)	6	15	2	5
Total	39	100	39	100

Discussion

Recently, a highly effective silica-based method for DNA extraction for STR analysis from skeletal remains obtained from mass graves was reported using the QIAGEN DNA Blood Maxi Kit with some modifications (13). In that report, the authors successfully obtained STR genetic profiles from exhumed bodies found in common graves. Their protocol was used for the analysis of 1823 bone or tooth samples from victims of the 2004 Asian tsunami (13). Because of the increasing need for STR profiles from human remains in Colombia, we decided to compare the proposed silica-based method to the organic extraction method that has been routinely used in our institution for the last 12 years, a protocol kindly provided by the Armed Forces DNA Identification Laboratory in 1997. Our aim was to standardize a DNA isolation method that would be faster to perform, would reduce the use of toxic reagents in the process, and could be used not only to isolate DNA from skeletal human remains for paternity testing purposes, but could also be used in future cases of biological relationship testing for identifying remains recovered from mass graves in Colombia. For that purpose, we used 4 g of bone per sample for the organic extraction method (as stated in our standard operating procedure) and a range of 2.4–6 g of bone per sample for the silica-based method. This was carried out to maintain similar amounts of bone used in accordance with the description of Davoren et al. (13)

We have had a success rate of 95–98% at obtaining complete STR plus amelogenin profiles using the organic extraction method throughout the years (data not shown) using either PowerPlex® 16 (Promega Corporation) or AmpF*l*STR® Identifiler® PCR amplification kits with similar efficiencies. The entire process usually takes

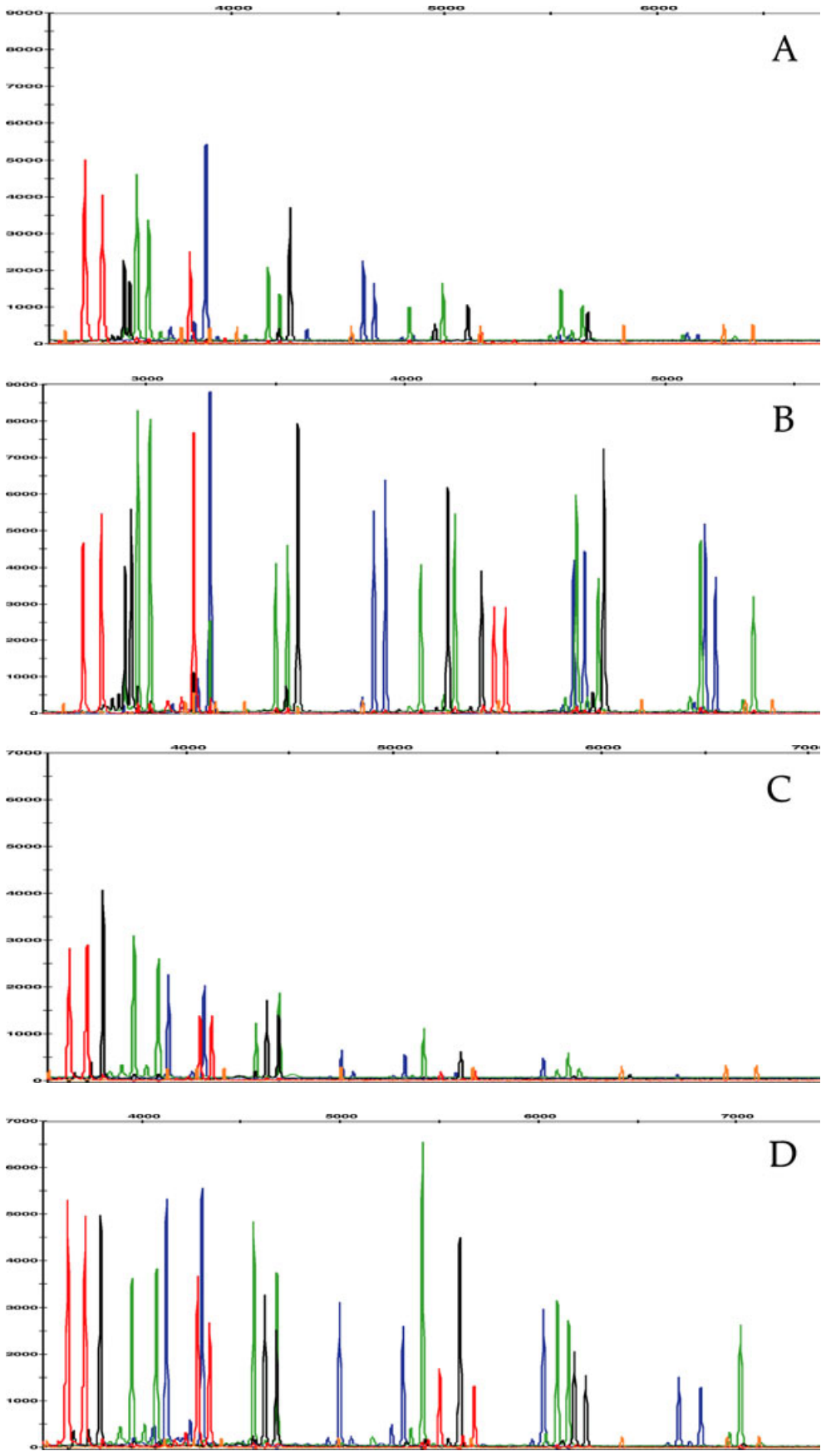

FIG. 1—*AmpFlSTR® Identifiler® profiles of DNA extracted from skeletal human remains using two different methods. Panel A refers to sample 105216′s profile derived from silica-based extracted DNA; Panel B refers to sample 105216′s profile derived from organically extracted DNA; Panel C refers to sample 108741′s profile derived from silica-based extracted DNA; and Panel D refers to sample 108741′s profile derived from organically extracted DNA. Notice that several loci failed to amplify in Panels A and C compared with Panels B and D.*

between 2 and 3 weeks depending on DNA quality and the amount of DNA obtained from each sample. In this study, we analyzed only AmpF*l*STR® Identifiler® because we wanted to minimize the variability of results by using the same lot number of reagents for all the samples tested. A faster method for isolating DNA could drastically reduce turnaround time, which is an important consideration in a country where the number of human remains requiring definitive identification is increasing. Current official data indicate that 32,682 individuals have been killed during the conflict (22).

One of the differences between the two methods used was that the powdered bone material completely dissolved in the organic extraction method, but not in the silica-based method. In fact, with the latter method, the majority of the samples remained undissolved. This result could account for the difference in the total amount of DNA isolated (ng/g of bone). Physical dissolution of bone powder is either achieved by soaking the bone powder in 0.5 M EDTA or by using a lysis buffer that includes EDTA as reported by others (16,24). Davoren et al. (13) have no EDTA decalcification step in their protocol, while it is a standard procedure in our methodology. Although it is claimed that DNA could be lost with each EDTA soaking step, in our results, this was not a factor because the yield of DNA was higher with the organic extraction method than with the silica-based method (median 5.8 vs. 1.3 ng/g, respectively). Quantitation by real-time PCR using the Plexor HY system showed a higher DNA concentration in samples isolated with the organic extraction method compared with the silica-based method, even when the latter method used more bone powder as the starting material for DNA isolation (Table 1). It is unlikely that the use of more bone powder in the silica-based method could adversely affect the recovery of DNA by saturating the silica column used. The manufacturer of the columns states that the columns used in this study (QIAGEN's Blood Maxi Kit) have a capacity for binding and recovering up to 600 μg of genomic DNA per column. The maximum amount of DNA recovered with this method was around 1 μg of DNA (sample 98169); therefore, there was plenty of binding capacity for the columns to capture DNA if it was present. The second difference between the previously reported silica-based method and the protocol used in our study was that a second DNA concentration step used by Davoren et al. (13) was not carried out in the present study. It is unlikely that this change could be responsible for the poorer outcome obtained with the silica-based DNA-isolation method because the final amount of DNA present in the retentate material contains the total amount of DNA present in each sample. A second concentration step might only help to eliminate particulate material if it was observed that the flow through the concentration membrane was obstructed. In such a case, we would expect to see significantly higher Ct values for the IPC with the silica-based method. However, similar Ct values for the IPC were obtained using both methods, ruling out this possibility. A third possible explanation for the poor outcome of the silica-based method could be the fact that a total of 4 mg of proteinase K/sample was used instead of the 10 mg used by Davoren et al. (13) It is possible that the use of less proteinase K could account for some of the differences. However, this is not the most plausible conclusion. Most of the samples that contained 5 g of bone processed with the silica-based method did not yield higher amounts of DNA than did the samples, which used 12 or less grams of bone for DNA isolation. Also, although the same amount of proteinase K was used to isolate samples with both methods, the amount of DNA recovered was greater from the organic extraction method than from the silica-based method. Thus, the most plausible conclusion is that the demineralization steps are required to expose the organic material from the inorganic matrix. This could explain why the silica-based method did not perform as well as the organic extraction method in our study.

Our results showed that the silica-based method as used did not provide better end results (a complete 15 STR profile and amelogenin) for the 39 samples tested than those obtained from the organically extracted DNA. In fact, while complete (16/16 loci) AmpF*l*STR® Identifiler® profiles were obtained in 95% of the organically extracted samples, only 23% of the samples obtained with the silica-based method gave a full profile. High partial profiles (8–15 loci) were obtained in 16/39 samples (41%), while low partial profiles were obtained in 8/39 samples tested (21%).

Three samples isolated with the organic extraction method gave a full AmpF*l*STR® Identifiler® profile with apparently no DNA based on the Plexor HY quantitation. The quantitation procedure was repeated to confirm the results. Also, full AmpF*l*STR® Identifiler® profiles were obtained in some samples with only a small amount of DNA based on the quantitation performed. Samples 103538, 109408, 100969 (organic extraction), and 101812 (silica based) amplified a full profile with as little as 40, 70, 100, and 140 pg of DNA, respectively. We have no clear explanation for these results. It is possible that PCR inhibitors may still been present in the DNA and that they affected the Plexor HY amplification but not the AmpF*l*STR® Identifiler® amplification. However, we cannot rule out pipetting errors because samples were not quantified in duplicate in the same experiment. There is no clear explanation for the differences between our results compared and those reported by Davoren et al. (13) except that the EDTA decalcification steps used in the organic extraction method significantly improved the amount of DNA recovered per gram of bone. In fact, our results with the silica-based method showed a similar recovered amount of DNA as did Davoren et al. (13). A median of 1.30 ng/g of DNA was obtained in the present study similar to the median of 1.49 ng/g of DNA reported in their study with the silica-based method (data derived from Table 1). Different conditions of soil composition, environmental temperature, humidity, and the presence of inhibitors (9,10,25) might be factors that affect the quality and quantity of DNA obtained. To rule out any factors during processing of samples, we used identical conditions for cleaning and pulverizing bone samples, and parallel processing for both methods. Therefore, the differences in the end results obtained could only be attributed to the different treatments used in both methods.

It could be claimed that the smaller amount of DNA used for AmpF*l*STR® Identifiler® amplification from samples isolated with the silica-based method could account for the smaller number of complete profiles (16 loci) obtained from these samples. However, in several of such samples, the amount of DNA used for the amplification contained more than the recommended amount of DNA by the manufacturer, yet no complete profiles were obtained. It is possible that PCR inhibitors are copurified and are still present along with the DNA. However, it is not clear to us why this would be the case in some instances and not in others in which similar DNA amounts were used for amplification. As an example, similar amounts of DNA were used for STR amplification from samples isolated with both methods (98169, 110179, 110846, and 108684), yet the efficiency of the two methods for obtaining complete profiles was very different.

Recently, a modification of the silica-based DNA extraction method that uses total demineralization has been published (16). In that report, the modification provided better results than those obtained with a silica-based method similar to the one reported in the ICMP report. These results further support the conclusion that demineralization is required to obtain quality DNA for generating

complete STR profiles from human remains. We are currently evaluating this modification to the protocol.

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References

1. Alonso A, Martín P, Albarrán C, García P. Challenges of DNA profiling in mass disaster investigations. Croat Med J 2005;46:540–8.
2. Lay T, Kanamori H, Ammon C, Nettles M, Ward S, Aster R, et al. The great Sumatra-Andaman earthquake of December 26, 2004. Science 2005;308:1127–33.
3. Zehner R. ''Foreign'' DNA in tissue adherent to compact bone from tsunami victims. Forensic Sci Int Genet 2007;1:218–22.
4. Schwartz SP, Li W, Berenson L, Williams RD. Morbidity and mortality weekly report (MMWR) deaths in World Trade Center terrorist attacks. New York City: CDC, 2001. September 11, 2002/51 (Special Issue);16–8, http://www.cdc.gov/mmwr/preview/mmwrhtml/mm51spa6.htm (accessed November 20, 2011).
5. Leclair B, Shaler R, Carmody GR, Eliason K, Hendrickson BC, Judkins T, et al. Bioinformatics and human identification in mass fatality incidents: the World Trade Center disaster. J Forensic Sci 2007;52:806–19.
6. Tindemans L. Declaration of The International Commission on the Balkans. Washington, DC: Aspen Institute Berlin and Carnegie Endowment for International Peace, August 6, 1997.
7. Palo JU, Hedman M, Söderholm N, Sajantila A. Repatriation and identification of the Finnish World War II soldiers. Croat Med J 2007;48:528–35.
8. Williams ED, Crews JD. From dust to dust: ethical and practical issues involved in the location, exhumation, and identification of bodies from mass graves. Croat Med J 2003;44:251–8.
9. Kaiser C, Bachmeier B, Conrad C, Nerlich A, Bratzke H, Eisenmenger W, et al. Molecular study of time dependent changes in DNA stability in soil buried skeletal residues. Forensic Sci Int 2008;177:32–6.
10. Misner LM, Halvorson AC, Dreier JL, Ubelaker DH, Foran DR. The correlation between skeletal weathering and DNA quality and quantity. J Forensic Sci 2009;54:822–8.
11. Miazato ES, Soares-Vieira J, Romero D. Human identification and analysis of DNA in bones. Rev Hosp Clín 2004;59:383–8.
12. Del Valle C, Rodríguez A, Espinoza M. Comparación de tres métodos de extracción de ADN a partir de restos óseos. Rev Biol Trop 2004;52:717–25.
13. Davoren J, Vanek D, Konjhodzic R, Crews J, Huffine E, Parsons TJ. Highly effective DNA extraction method for nuclear short tandem repeat testing of skeletal remains from mass graves. Croat Med J 2007;48:478–85.
14. Holland M, Cave C, Holland C, Bille T. Development of a quality, high throughput DNA analysis procedure for skeletal samples to assist with the identification of victims from the World Trade Center attacks. Croat Med J 2003;44:264–72.
15. Anderung C, Persson P, Bouwman A, Elburg R, Götherström A. Fishing for ancient DNA. Forensic Sci Int Genet 2008;2(2):104–7.
16. Lee HY, Park MJ, Kim NY, Sim JE, Yang WI, Shin KJ. Simple and highly effective DNA extraction methods from old skeletal remains using silica columns. Forensic Sci Int Genet 2010;4(5):275–80.
17. International Commission on Missing Persons. The ICMP Report: Colombia′s response to enforced disappearances. Sarajevo 2008. (ICMP.Cos.110.3. Doc from April 2008), http://www.ic-mp.org/wp-content/uploads/2008/10/icmp-cos-110-3-doc.pdf (accessed April 15, 2010).
18. Pécaut D. Orden y violencia: Colombia, 1930–1954. Bogotá: ed. Siglo XXI-Cerec, 1987.
19. Guzmán G, Fals O, Umaña E. La Violencia en Colombia: estudio de un proceso social. Bogotá: ed. Círculo de Lectores, 1988.
20. Comisión de Estudios sobre la Violencia. Colombia: violencia y democracia. Informe presentado al Ministerio de Gobierno, Bogotá. Centro de Estudios Sociales. Colombia: Universidad Nacional de Colombia, Colciencias, 1988.
21. Arias FJ, Muñoz J. Desaparición forzada y exhumaciones. Fundación Dos Mundos. Bogotá: ed. Fundación Dos Mundos, 2008, http://www.dos-mundos.org/Documento%20CICR%20final.pdf (accessed April 15, 2010).
22. Fiscalia General de la Nación. República de Colombia. Justicia y Paz. 2010, http://www.fiscalia.gov.co/justiciapaz/index.htm (accessed June 30, 2010).
23. Global Peace Index. 2008 methodology, results & findings. Institute for Economics & Peace, 2008, http://www.visionofhumanity.org/gpi-data/#/2008/scor (accessed April 15, 2010).
24. Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. Forensic Sci Int Genet 2007;1:191–5.
25. Mundorff AZ, Bartelink EJ, Mar-Cash E. DNA preservation in skeletal elements from the World Trade Center disaster: recommendations for mass fatality management. J Forensic Sci 2009;54:739–45.

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