DNA extraction from human bone: comparison of magnetic bead and silica column techniques

Extração de DNA de ossos humanos: comparação entre as técnicas de partículas magnéticas e coluna de sílica

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ABSTRACT

Objective: The present study aimed the comparison between the automated magnetic bead and silica column techniques for DNA extraction of human bones. Material and methods: Both techniques were performed on 25 human femur bones evaluating: I) the amount of extracted genetic material; II) the amount of amplified profiles; and III) the necessary time range to perform the techniques. Results: The automated magnetic bead technique recovered larger amount of DNA in 88% of the studied bone sample in comparison with the silica column technique. The automated magnetic bead technique also achieved a high level of amplifications (9/16 loci) in 68% of the sample, while the silica column technique reached equal level of amplifications only in 36% of the sample. The time range elapsed for performing the automated magnetic bead technique was approximately 3 hours for processing 12 samples, while the silica column technique performed the same samples in 81 hours. Conclusion: Based on that, the automated magnetic bead technique presented optimal outcomes and faster performance compared to the silica column technique, revealing a valuable tool for forensic DNA extraction.

KEYWORDS: Magnetic bead; Silica column; Forensic genetics; DNA extraction; Bone.

INTRODUÇÃO

Human identification through dactyloscopy and odontology is commonly hampered in mass disaster environments, in which soft tissues are often destroyed[1] and dental records may be absent[2]. In this context, forensic genetics arises as a valuable alternative for the post-mortem (PM) examination of human remains[3]. Specifically, the human bone is highly resistant to cadaveric alterations, becoming an excellent source for PM DNA extraction[4-6]. On the other hand, about 70% of the human bone consists of inorganic material, representing a physical barrier for DNA extraction reagents[7]. Additionally, forensic genetics is also limited due to degraded DNA and inhibitors of Polymerase Chain Reaction (PCR)[8,9]. Thus, problem-based researches are developed to enhance and standardize DNA extraction from human bones[4,7,10-14].

Initially, Hochmeister et al.15 (1991), reported the forensic extraction of DNA from the human femur bone. The addressed methodology for DNA extraction was based on the partial decalcification, cell lysis and separation of DNA using phenol-chloroform. Further studies on the field followed the traditional methodology[4,16,17]. On the other hand, Rohland and Hofreiter[13] (2007), stated that chemical reagents, such as the phenol-chloroform, lack the efficient removal of PCR inhibitors; promote chemical toxicity; and require a high amount of sample for a proper analysis. Moreover, the traditional methodology is manually performed and more susceptive to subjectivity[12].

Recently, several bioassays were developed and validated in forensic genetics in order to improve the DNA extraction and analysis[18-21]. Mainly, these assays approach two pathways for DNA extraction: the automated magnetic bead and the silica column techniques. Based on that, the present study aims to compare these two techniques evaluating: I) the amount of extracted DNA material; II) the amount of amplified DNA profiles; and III) the necessary time range for technical performance.

MATERIAL AND METHODS

The sample consisted of 25 human femurs from deceased victims who underwent human identification through DNA analysis at the Laboratory of DNA of the Scientific Police of Goiás between the years of 2006 and 2011. No information about gender and age of the victims were available.

The sample underwent initial cleaning process using Extran® (Merck KGaA®, Darmstadt, Germany) solution; hypochlorite solution 0,3%; and distilled water. The surface of each femur bone was sanded using a Dremel® 100 series rotary tool (Robert Bosch Tool® Corp., Wisconsin, USA), and a portion of approximately 3g was sectioned from each femur bone for analysis. Further on, the obtained bone portions were frozen at -80ºC per 24 hours and ground using an Ika® A11 basic analytical mill (Ika Works® Inc., North Carolina, USA).

Twenty-five aliquots of 100mg of powdered sample bone were submitted for DNA extraction through two different techniques: I) the automated magnetic bead, using the PrepFiler® BTA forensic DNA extraction Kit (Life Technologies® Corp., California, USA), combined with the AutoMate Express® forensic DNA extraction system (Life Technologies® Corp., California, USA); and II) the column of silica approach, manually performed, using the QIAamp® DNA Investigator Kit (Qiagen®, Venlo, Netherlands). The procedures for both methods were performed according to the manufacturer's ins-

tructions. After DNA extraction, the samples were quantified by real time PCR, using the Plexor® HY system (Promega®, Wisconsin, USA) and a IQ5® (Bio-Rad®, California, USA) thermal cycler. The presence of PCR inhibitors was assessed using the Internal PCR Control (IPC).

The DNA samples were amplified through PCR using the AmpFlSTR® Identifiler® Plus Kit (Life Technologies® Corp., California, USA). The final reaction volume (sample + reaction mix) was 25uL. The samples with DNA concentrations below the manufacturer's requirements indicated in the used kit were normalized for amplification. So, reamplification was performed using AmpFlSTR® MiniFile® PCR (Life Technologies® Corp., California, USA) and AmpFlSTR® NGM® amplification kits (Life Technologies® Corp., California, USA). All the amplifications were performed in a GeneAmp® PCR System 9700 thermal cycler (Life Technologies® Corp., California, USA). The number of cycles; time of incubation; and amount of reagents followed the manufacturer's instructions.

The amplified DNA underwent capillary electrophoresis in a 3130xL genetic analyzer (Life Technologies® Corp., California, USA) and the obtained data was analyzed using the GeneMapper® ID software (Life Technologies® Corp., California, USA).

RESULTS

Table 1 reports the amount of DNA extracted from each of the 25 samples using both extraction techniques. In 22 bone samples, the concentration of extracted DNA was higher using the automated magnetic bead technique than the silica column technique. Specifically, the automated magnetic bead technique provided up to four times more DNA than the other technique. Both techniques did not reveal PCR inhibitors through IPC analysis.

Table 1 also relates the outcomes from DNA amplification considering the number of amplified loci, from a total of 16 markers (Table 1). In 24 bone samples, the number of amplified loci using the automated magnetic bead technique was equal or higher to the number of amplified loci through silica column technique. Specifically, the magnetic bead technique resulted in 10 complete genetic profiles (16/16 loci), while the silica column technique achieved only 5 complete profiles. Moreover, the magnetic bead technique amplified ≥9/16 loci in 68% of the studied sample, while the silica column reached only 36% (Table 2).

The time range for extraction procedure was delimited from the incubation, placing the lysis buffer, to the final DNA extraction. The magnetic bead technique took approximately 3 hours to extract the DNA from 12 femur bones, while 81 hours were necessary to extract the DNA from the same samples using the silica columns technique.

DISCUSSION

The use of alternative, non-toxic, genetic kits for DNA extraction is explored in the medical literature. Mostly, the advantages of these alternative extraction techniques are related to the elimination of potential PCR inhibitors, and the feasibility of performing automated, and more objective, laboratory procedures[10,18-20]. The present study aimed the investigation of two alternative techniques for DNA extraction in face of the amount of extracted material; the amount of amplified profiles; and the time range necessary to perform both techniques. Specifically, the automated magnetic bead technique, using the PrepFiler® BTA forensic DNA extraction kit (Life Technologies® Corp., California, USA), revealed improved outcomes considering the amount of extracted DNA material if compared to the silica column technique. Mainly, it is justified due to the especial formulation in which the extraction kit was developed. In detail, the addressed forensic kit is highly applicable for critical sampling, such as DNA extraction from bones and teeth. Additionally, the incubation procedure with lysis buffer is carried in a thermo shaker, enabling a homogeneous shaking under constant temperature, consequently allowing a greater contact between the reagent and the target cells.

The present study also stressed the amount of PCR inhibitors after DNA extraction through the different techniques. It was performed based on the hypothesis that specific forensic samples, such as

Table 1 - Results of quantitative real-time PCR and STR analysis comparing different DNA extraction techniques for 25 samples of the human femur

Sample ID	Magnetic bead		Silica column	
	pg/uL	STR profile*	pg/uL	STR profile*
LD 02-07	08.76	9	05.78	9
LD 07-07	03.00	8	01.03	2
LD 67-07	50.00	16	12.70	16
LD 03-08	01.48	11	07.10	4
LD 12-08	07.72	-	01.49	2
LD 102-08	24.80	16	06.81	5
LD 121-08	17.50	16	05.53	1
LD 22-09	122.0	16	38.80	16
LD 34-09	874.0	16	263.0	16
LD 54-09	01.91	-	-	-
LD 61-09	10.80	15	07.51	2
LD 135-09	21.20	5	09.59	11
LD 02-10	68.20	16	18.00	13
LD 36-10	06.98	13	03.13	4
LD 67-10	11.90	8	04.05	-
LD 68-10	07.83	13	02.40	9
LD 84-10	03.03	-	01.16	-
LD 119-10	17.30	16	13.20	16
LD 122-10	51.90	16	49.90	16
LD 62-11	04.64	-	02.60	-
LD 65-11	02.67	9	01.89	-
LD 77-11	01.16	-	02.47	-
LD 127-11	09.07	16	02.39	-
LD 151-11	08.08	16	03.54	4
LD 307-11	05.04	9	05.35	2

* Number of amplified loci.

Table 2 – Rates of STR profiles obtained through magnetic bead and silica column techniques.

PCR profile	Magnetic bead		Silica column	
	Nº	%	Nº	%
Complete profile (16 loci)	10	40	5	20
Large profile (≥ 9 loci)	7	28	4	16
Small profile (< 9 loci)	3	12	9	36
No profile (0 loci)	5	20	7	28
Total	25	100	25	100

the human bone, are often degraded and potentially contains PCR inhibitors[22]. The main factors affecting the PCR procedure in DNA samples from bones are the quantity of extracted nuclear DNA and its respective degradation[9]. However, in the present study both techniques eliminated PCR inhibitors, decreasing the IPC values, indicating the extraction of pure DNA samples.

The second aim of the present research concerned the investigation of amplified profiles. Once more, the automated magnetic bead technique revealed better outcomes, achieving higher amplifications in most of the bones samples. Accordingly, other authors detected the same enhanced performance of the magnetic bead technique producing full profiles (16/16 loci) from pure DNA of forensic samples[23,24].

Finally, the time range necessary for technical performance was also approached in the present research. A greater advantage was observed by performing the automated magnetic bead technique in face of the silica column technique. This finding is justified due to the automated design in which the magnetic bead technique was developed. Specifically, this technique enables the simultaneous manipulation of 13 samples[23,24], consequently enhancing the practical routine of forensic laboratories. Our results corroborate the literature, once the automated technique required 3 hours in order to extract the DNA from 12 samples, while the silica column technique took approximately 81 hours to extract the DNA from the same samples. It reveals a useful application in mass disaster situations, in which the demand for fast and accurate human identification is observed. Despite the great advantages of using the automated magnetic bead technique in the forensic practice, a relevant limitation, such as the high cost to afford the necessary equipment, remains. Thus, the reproduction of this technique in less developed laboratories may be hampered.

CONCLUSION

In summation, the automation of DNA extraction optimizes the forensic routine reducing the necessary time for data analysis and the error induced by human influence. In the present study, two different techniques were compared in order to stress the best alternative for forensic applications based on practical evidences. In this context, the magnetic bead technique revealed improved applications for forensic purposes in face of the silica column approach. Specifically, this technique is highly useful in mass disaster environments, in which fast and accurate analyses for human identification are necessary. On the other hand, the automated system associated with the magnetic bead technique may not be available in some forensic facilities due to the high cost of equipment.

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RESUMO

Objetivo: O presente objetiva comparar as técnicas de partículas magnéticas e de coluna de sílica para a extração de DNA a partir de ossos humanos. Materiais e métodos: Ambas as técnicas foram aplicadas em 25 fêmures humanos, visando avaliar: I - a quantidade de material genético extraído; II - a quantidade de material amplificado; e III - o tempo decorrido para a aplicação de cada técnica. Resultados: A técnica de partículas magnéticas viabilizou maior quantidade de material extraído em 88% da amostra. A mesma técnica alcançou também maior quantia de material amplificado (9/16 loci) em 68% da amostra. O tempo decorrido para a aplicação da técnica de partículas magnéticas consistiu num período de 3 horas para o processamento de 12 amostras, enquanto a técnica de coluna de sílica realizou o mesmo procedimento em 81 horas. Conclusão: Desta forma, a técnica de partículas magnéticas apresentou resultados mais satisfatórios dentro de um desempenho mais rápido quando comparada com a técnica de coluna de sílica, revelando ser uma eficaz ferramenta forense.

PALAVRAS-CHAVE: Partículas magnéticas; Coluna de sílica; Genética Forense; Extração de DNA; Osso.

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